Biochem. J. (1978) **174**, 663–666
Printed in Great Britain

The Lipoprotein Lipase (Clearing-Factor Lipase) Activity of Cells Isolated from Rat Cardiac Muscle

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(*Received* 11 *May* 1978)

The total lipoprotein lipase activity recovered in suspensions of cells prepared from adult rat hearts was unaffected by the nutritional state of the animals used. The enzyme activity present in the cell suspensions was almost exclusively associated with the cardiac muscle cells present as the major cell type.

Lipoprotein lipase (clearing-factor lipase; EC 3.1.1.3) regulates the removal of lipoprotein triacylglycerol fatty acids from the plasma by the extrahepatic tissues (Robinson, 1970). Considerable direct and indirect evidence exists for the presence of the enzyme both in the adipocyte and at the endothelial cell surface in adipose tissue (Cryer *et al.*, 1975; Scow *et al.*, 1976). Less information exists concerning the distribution of the enzyme among the cell types present in the heart. In this organ one way of investigating this problem has been to estimate the proportion of total cardiac lipoprotein lipase activity that is rapidly released by heparin and therefore presumed to be at or near the endothelial cell surface (Borensztajn & Robinson, 1970; Fielding & Higgins, 1974; Rogers & Robinson, 1974; Borensztajn *et al.*, 1975). However, although lipoprotein lipase has been reported to be present in cultured cells derived from neonatal hearts (Pinson *et al.*, 1973; Lindsey *et al.*, 1977) and has been shown to be present in more than one cultured cell type from such hearts (Chajek *et al.*, 1978*a*,*b*), only one preliminary report has indicated the presence of the enzyme in isolated cardiac muscle cells derived from the hearts of adult animals (Bagby *et al.*, 1977).

In the present paper, data derived from isolated-cell studies and enzyme-stability measurements as previously applied to adipose tissue (Cunningham & Robinson, 1969; Davies & Robinson, 1973) and intact heart tissue (Chohan & Cryer, 1977) have been used. We report evidence in support of the view that lipoprotein lipase is present at more than one site in adult heart tissue and that the cardiac muscle cell is an important one of these.

Materials and Methods

Animals

Male rats of the M.R.C. Hooded strain, from the colony of this department, were used throughout. They were maintained on Modified Diet 41B (Pilsbury and Co., Birmingham, U.K.) and weighed 190–210g in the fed state. Animals were killed between 08:00 and 09:30h. Starved animals were deprived of food 24h before use.

Preparation of isolated cells

Cells were prepared from the hearts of either fed or 24h-starved rats by a modification of the procedure of Powell & Twist (1976). For this, eight hearts were perfused simultaneously with medium containing collagenase [type II; Sigma (London) Chemical Co., Kingston upon Thames KT2 7BH, U.K.; 0.5mg/ml] for 20min in an apparatus similar to that described by Farmer *et al.* (1977). After perfusion, 1mm slices of the softened heart tissue were incubated (four hearts per flask) in 30ml of fresh medium (Powell & Twist, 1976) containing fresh collagenase (0.5mg/ml) and 2% (w/v) fatty acid-poor bovine serum albumin (Sigma). The incubation was continued at 37°C, in an atmosphere of O_2/CO_2 (19:1), until tissue disruption was complete (50–10min). The cell suspension was then filtered through nylon mesh (0.13mm diameter) and centrifuged for 3–4min at $100g_{av.}$. The cells were washed free of enzymes and debris by using the procedure of Powell & Twist (1976) except that the force applied during centrifugation was increased to $100g_{av.}$. In some preparations the non-muscle cells, present as a small proportion by mass of these suspensions, were removed by density-gradient centrifugation by using the method of Cutilletta *et al.* (1977). The viability of isolated cells was assessed by Trypan Blue exclusion (Howard & Pesch, 1968). Typically the percentage of viable cells present in the preparations was 60%. Dry weight and wet weight of cells were determined as described by Farmer *et al.* (1977). Typically the wet weight of cells recovered represented 20–30% of the weight of the original tissue.

Incubations

Krebs–Ringer bicarbonate buffer, pH7.4 (154mM-NaCl/154mM-KCl/55mM-$CaCl_2$/280mM-KH_2PO_4/

154mM-$NaHCO_3$), containing 4% (w/v) bovine serum albumin, and 50mM-NH_3/NH_4Cl buffer, pH 8.1, were both used as incubation media in the study of cell enzyme stability. For this the cells from eight hearts were suspended in 9ml of medium and incubated at 4°C for up to 8h. During such incubations and during cell preparation the lipoprotein lipase activity present was found exclusively associated with cells and was not detected in the media. After incubation homogenates of cells in the incubation medium were used in the assay of enzyme activity. Cells and acetone/diethyl ether-dried powders of cells, prepared by the method of Cunningham & Robinson (1969) and homogenized at 56–65mg fresh weight of cells/ml, were also prepared and incubated at 42°C for up to 8h in the media described. Portions of these homogenates were used directly in the assay of enzyme activity.

Enzyme activity measurements

Total lipoprotein lipase activities were determined as the rate of non-esterified fatty acid release from an apolipoprotein C-II-activated triacylglycerol emulsion (Intralipid; Vitrum, Stockholm, Sweden) at 30°C as described by Cryer & Jones (1978). The enzyme activity is expressed as μmol of non-esterified fatty acid released/h per g wet weight of tissue or cells. The enzyme was characterized in all the preparations by the degree of inhibition (>90% in all cases) observed when 0.6M-NaCl was present in the assay and by the obligatory requirement for activity of serum in the assay. Ca^{2+}-stimulated myosin adenosine triphosphatase activities were determined by the method of Perry (1955).

Protein was measured by the method of Lowry *et al.* (1951). Activities are expressed as means ±S.D. with the numbers of observations in parentheses Student's *t* test (Fisher & Yates, 1957) was used to assess the significance of the differences between means.

Results and Discussion

The modifications in the procedure described above produce, in relatively high yield, a suspension of intact cells from adult rat heart that is rich in cardiac muscle cells. The total lipoprotein lipase activity (units/g wet weight of cells) recovered in these suspensions of washed cells was the same for those prepared from hearts taken from either fed or 24h-starved animals (Table 1). This is in parallel with the comparative recovery of enzyme activity in adipocytes prepared from the adipose tissue of such animals (Cunningham & Robinson, 1969) and in contrast with the difference in activity measured in whole cardiac or adipose tissue when fed and starved animals are compared (Table 1) (Cunningham & Robinson, 1969). This distinction was apparent when either fresh material or acetone/diethyl ether-dried powders of tissue or isolated cells were used in the assay (Table 1). The difference between the activities measured in homogenates of fresh cells and homogenates of acetone/diethyl ether-dried powders of cells prepared from animals in a particular nutritional state was greater (4–5-fold) than the corresponding difference observed with whole-tissue preparations (1.5-fold) ($P < 0.001$). This enhancement of activity in acetone/diethyl ether-dried powder preparations has been reported previously for whole heart tissue (Borensztajn & Robinson, 1970), but, like the difference in response between cells and tissue, remains unexplained.

When cell suspensions were subjected to density-

Table 1. *Comparison of the lipoprotein lipase activity determined in whole heart tissue and isolated cell suspensions of fed and 24h-starved rats*

Hearts from either fed or 24h-starved rats were assayed for lipoprotein lipase activity after either homogenization (*a*) or acetone/diethyl ether-dried preparation (*b*). Hearts from similar animals were used in the preparation of isolated cell suspensions as described in the Materials and Methods section. Enzyme activity was determined in aqueous homogenates of these cells (*a*) or in homogenates of acetone/diethyl ether-dried powders of cells (*b*). The activities are given as means ± S.D. with the numbers of independent preparations studied in parentheses. The enzyme activity in each preparation was shown to be >90% inhibited by 0.6M-NaCl and to be dependent on serum for maximal rates of hydrolysis. The homogenization medium was 50mM-NH_3/NH_4Cl, pH 8.1, in all cases.

	Lipoprotein lipase activity (μmol of non-esterified fatty acid released/h per g of tissue or cells)			
	Fed animals		24h-starved animals	
Preparation used in assay	Whole tissue	Cells	Whole tissue	Cells
(*a*) Homogenate of fresh material	38.3 ± 1.8 (10)	11.8 ± 1.3 (12)	84.0 ± 9.8 (16)	13.2 ± 2.3 (9)
(*b*) Homogenate of acetone/diethyl ether-dried powder	58.9 ± 4.5 (6)	61.1 ± 16.3 (6)	120.3 ± 2.9 (6)	56.4 ± 8.4 (6)
(*b*)/(*a*)	1.5	5.2	1.4	4.3

gradient centrifugation as described in the Materials and Methods section two distinct fractions were obtained. The major fraction, which contained 70% of the protein present in the original suspension and contained 93% of the total Ca^{2+}-dependent myosin adenosine triphosphatase activity, was considered to be a muscle-cell-enriched fraction. This major muscle-cell-enriched fraction contained $90 \pm 7\%$ (4) of the total lipoprotein lipase activity present in the original suspension. Only $10 \pm 7\%$ (4) of the lipoprotein lipase activity of the original suspension was recovered in the minor cell fraction characterized by its total lack of Ca^{2+}-stimulated myosin adenosine triphosphatase activity. This observation, together with the specific activities (units/mg of protein) of lipoprotein lipase present in the two cell fractions (0.23 for the muscle cells and 0.07 for the non-muscle cells), which are distinct from the differences reported for cultured cells (Chajek *et al.*, 1978*a*,*b*), indicate the quantitatively important contribution to total tissue lipoprotein lipase activity made by muscle cells from adult rat hearts and further indicate the relatively small contribution made by non-muscle cells after collagenase digestion of the organ (see also Cunningham & Robinson, 1969).

When intact heart slices were incubated at 4°C the total activity of lipoprotein lipase stable to the treatment was the same for slices from both fed and starved animals (Chohan & Cryer, 1977) and was similar to the total activities recovered in the cell suspensions described here (Table 1). This, together with the observation that enzyme activity associated with the isolated cell suspensions was stable to prolonged incubation, provided the cells remained intact (Fig. 1), indicates that in heart tissue lipoprotein lipase exists at more than one site in the tissue. In that the total activity within the muscle cells remains constant during changes in nutritional status, it must be that amount of enzyme activity, labile to both heat and collagenase exposure, outside these cells that changes with alteration in nutritional state and is reflected by changes in total tissue activity. This situation echoes in many regards that revealed by a similar experimental approach in adipose tissue (Cunningham & Robinson, 1969; Davies & Robinson, 1973).

The loss of cellular lipoprotein lipase activity during incubation of broken heart-cell preparations is as previously reported for adipocytes (Davies & Robinson, 1973), but the relative enhancement of stability in aqueous homogenates of heart cells in NH_3/NH_4Cl buffer remains unexplained.

The present studies suggest that lipoprotein lipase activity in heart tissue exists in at least two tissue sites, one of these being within the tissue muscle cells. There seems little evidence for a quantitatively important pool of enzyme activity to exist within non-muscle cells, that is distinct from that extracellular enzyme activity present at the surface of endothelial cells, in

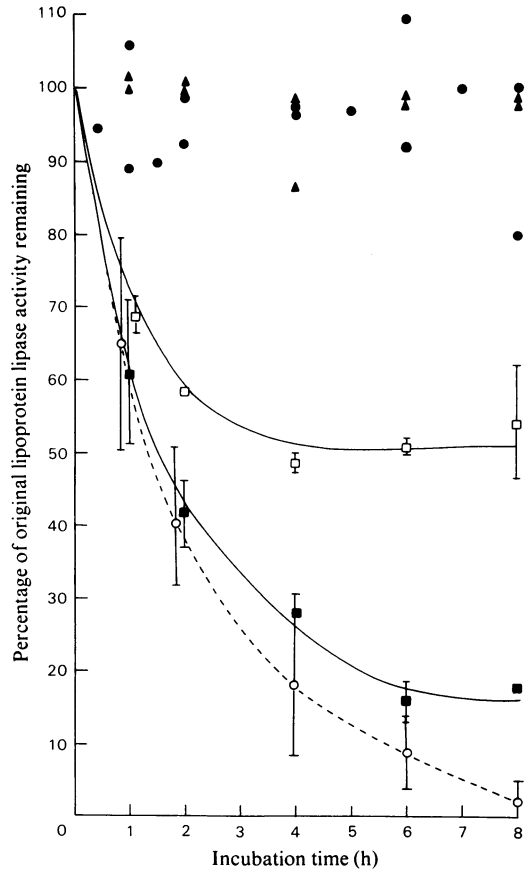

Fig. 1. *Changes in lipoprotein lipase activity during incubation of intact isolated cardiac muscle cells and homogenates of cells*

Isolated cardiac muscle cells were prepared as described in the Materials and Methods section from fed or 24h-starved rats and were incubated either in Krebs–Ringer bicarbonate buffer, pH7.4, containing 4% (w/v) bovine serum albumin (●), or in 50mM-NH_3/NH_4Cl buffer, pH8.1 (▲). No activity was detected in the media alone after incubation with cells. Each point indicates a duplicate estimate on a distinct cell preparation. Homogenates of cells from either fed or starved rats were prepared in either the Krebs–Ringer (■) or NH_3/NH_4Cl buffer (□) and also incubated at 42°C. The cells from eight hearts were homogenized in 9ml of medium, 1ml samples being used for enzyme assay. Acetone/diethyl ether-dried powders of cells from eight hearts of either fed or starved rats were prepared as described in the Materials and Methods section and separate powders homogenized in either of the incubation buffers. These homogenates were incubated at 42°C and changes in enzyme activity followed (○). Each point indicates the mean of four observations on distinct homogenates. The bars indicate ± S.E.M.

the adult heart. The observations are consistent with the view that the changes in total tissue activity that occur in response to nutritional and hormonal changes represent changes in the amount of enzyme outside the muscle cells from which the enzyme may be exported (Chajek *et al.*, 1978*a*,*b*).

We thank the British Heart Foundation for a grant in support of part of this work.

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