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Short-term incubation of cardiac myocytes with isoprenaline has no effect on heparin-releasable or cellular lipoprotein lipase activity

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Heparin (5 units/ml) produced a rapid (5–10 min) release of lipoprotein lipase (LPL) into the incubation medium of cardiac myocytes. Preincubation of myocytes for 30 min with 0.01–10 μM-isoprenaline, 100 μM-forskolin or 500 μM-8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate did not increase heparin-releasable LPL activity. Incubation with isoprenaline also did not change cellular LPL activity, even though the catecholamine did increase the phosphorylase *a* activity ratio.

INTRODUCTION

Lipoprotein lipase (LPL) is located at the capillary endothelial cell surface in the heart [1], where it catalyses the hydrolysis of the triacylglycerol component of circulating lipoproteins [2,3]. LPL is not synthesized in endothelial cells, but rather is translocated to the capillary endothelium after synthesis, processing and secretion from other tissue cells [2,3]. Consequently, LPL activity can still be detected in heart homogenates after perfusion with heparin to remove the endothelium-bound enzyme, and is referred to as 'residual LPL' [4]. It has not been established unequivocably which cardiac tissue cell(s) contain this residual LPL that will be the precursor of the functional enzyme at the capillary endothelium. LPL is present in cultured mesenchymal cells from newborn-rat hearts [5–7] and in isolated myocardial cells from adult-rat hearts [8–10]; Chohan & Cryer [11] have reported that all LPL activity in whole heart preparations from fed rats was recovered in cardiac myocytes. The secretion of LPL from cultured heart mesenchymal cells [5–7] and cardiac myocytes [12–14] can be induced by the addition of heparin to the incubation medium. Friedman *et al.* [7] have reported that incubation of cultured mesenchymal heart cells with isoprenaline (isoproterenol) for 30 min produced a 3-fold increase in heparin-releasable LPL activity, measured 3 min after addition of heparin to the culture medium. Our objective, therefore, was to determine the effect of isoprenaline and other interventions that increase cyclic AMP on heparin-releasable LPL activity, using cardiac myocytes isolated from adult rat hearts. Palmer & Kane [15] have shown that incubation of cardiac myocytes with adrenaline for 15 min resulted in a 35% increase in an intracellular lipase activity that has the characteristics of LPL, and so the effect of isoprenaline on cellular LPL activity was also investigated.

MATERIALS AND METHODS

Ca^{2+}-tolerant myocytes were isolated from the hearts of adult male rats as described by Kryski *et al.* [16]. The viability of myocytes, assessed as the percentage of rod-shaped cells that excluded Trypan Blue, ranged from 75 to 90%. In experiments to measure heparin-releasable LPL activity, myocytes were suspended in Joklik minimum essential medium containing 1.5 mM-$CaCl_2$ and 1% (w/v) defatted albumin [16] to a density of approx. 4×10^5 cells/ml, and were incubated at 37 °C under O_2/CO_2 (19:1). After addition of heparin (Organon Canada Ltd., Toronto, Ont., Canada) to a final concentration of 5 units/ml, 1 ml samples were removed after 5 or 10 min of incubation, unless noted otherwise, and were centrifuged for 10 s at 15000 *g* in an Eppendorf micro-centrifuge. The medium was decanted into tubes and frozen at −80 °C; the corresponding cell pellet was frozen by placing the micro-centrifuge tube in dry ice and was also stored at −80 °C.

Cell pellets were resuspended in 1 ml of a sucrose buffer (0.25 M-sucrose/1 mM-EDTA/1 mM-dithiothreitol/10 mM-Hepes, pH 7.4) by sonication for 6 × 30 s (BraunSonic 1510; 75 W). Samples of either the medium or the sonicated cells were then assayed for LPL activity with a [^{3}H]triolein (glycerol tri[9,10(n)-^{3}H]oleate) substrate preparation as described previously [10,14]. In some experiments, as noted in the text, myocytes were preincubated with iosproenaline (0.01–10 μM), forskolin (100 μM; Calbiochem–Behring, La Jolla, CA, U.S.A.) or 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (500 μM; Boehringer–Mannheim, Dorval, Que., Canada), for 30 min before the addition of heparin. For the determination of cyclic AMP concentrations, $HClO_4$ was added to 1 ml portions of myocyte incubation mixtures, to a final concentration of 5% (v/v). After deproteinization and neutralization, cyclic AMP content was measured by radioassay (Amersham Canada Ltd., Oakville, Ont., Canada).

The effect of isoprenaline on cellular LPL activity and on the phosphorylase *a* activity ratio was examined in experiments where myocytes were suspended (10^6 cells/ml) in a Krebs–Henseleit bicarbonate buffer [16]. Myocytes were then incubated in the absence and in the presence of 10 μM-isoprenaline for 15 min at 37 °C under

Abbreviation used: LPL, lipoprotein lipase.

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O_2/CO_2 (19:1). Two methods were utilized for preparing cell extracts for enzyme assays. In procedure A, duplicate 1 ml samples were removed from incubations and were centrifuged for 10–15 s at 15000 *g* in an Eppendorf micro-centrifuge with a pre-cooled rotor. The supernatants were discarded and the cell pellets were quickly frozen in liquid N_2 or in a solid-CO_2/ethanol mixture. One of the cell pellets was then resuspended in sucrose buffer by sonication, and LPL activity was measured as noted above. The second cell pellet was resuspended by sonication into a solution consisting of 40 mM-glycerol phosphate, 40 mM-2-mercaptoethanol, 10 mM-NaF and 0.1% (w/v) albumin, pH 6.8. The myocyte homogenate was centrifuged at 1000 *g* for 5 min, and phosphorylase *a* activity (with or without 2 mM-AMP) in the supernatant was measured by the procedure of Hardman *et al.* [17]. In procedure B, duplicate 1 ml portions of the incubation medium were transferred to tubes containing 3 ml of cold acetone (−20 °C), and acetone/diethyl ether-dried powders were prepared [14]. The powders were then suspended into the respective buffers for LPL and phosphorylase *a* assays as described above.

RESULTS

The addition of heparin resulted in the rapid release of LPL into the incubation medium of cardiac myocytes (Fig. 1), as observed previously by Ramírez & Severson [14]. The lipase activity released by heparin was completely serum-dependent (Fig. 1), and serum-stimulated lipase activity in the medium could be completely inhibited by the addition of 1 M-NaCl to the assay (results not shown). Little or no serum-dependent lipase activity was released into the medium during incubations in the absence of heparin. In subsequent experiments, the release of LPL was routinely measured after 5 or 10 min incubations of myocytes with heparin.

Preincubation of myocytes for 30 min with concentrations of isoprenaline from 0.01 to 10 μM had no effect on the basal release of LPL (pre-heparin) or on heparin-releasable LPL activity (post-heparin; Fig. 2*a*). In four experiments, the heparin-releasable LPL activity determined 5 min after addition of heparin to myocytes preincubated for 30 min in the absence and in the presence of 10 μM-isoprenaline was 219±36 and 244±48 nmol/h per 10^6 cells respectively (means± S.E.M.).

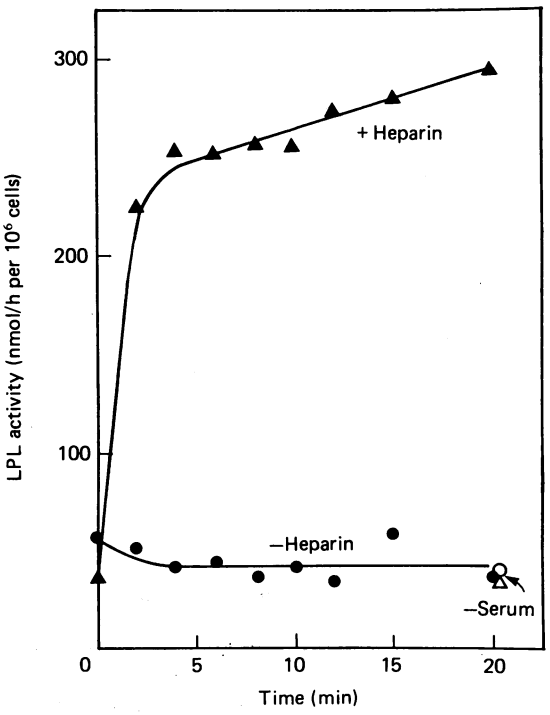

Fig. 1. Effect of heparin on the release of LPL from cardiac myocytes

Myocytes were incubated in the absence (○, ●) and in the presence (△, ▲) of heparin (5 units/ml). At the indicated time intervals, lipase activity in the medium after centrifugation was measured in the presence (●, ▲) and in the absence (○, △) of 2% serum.

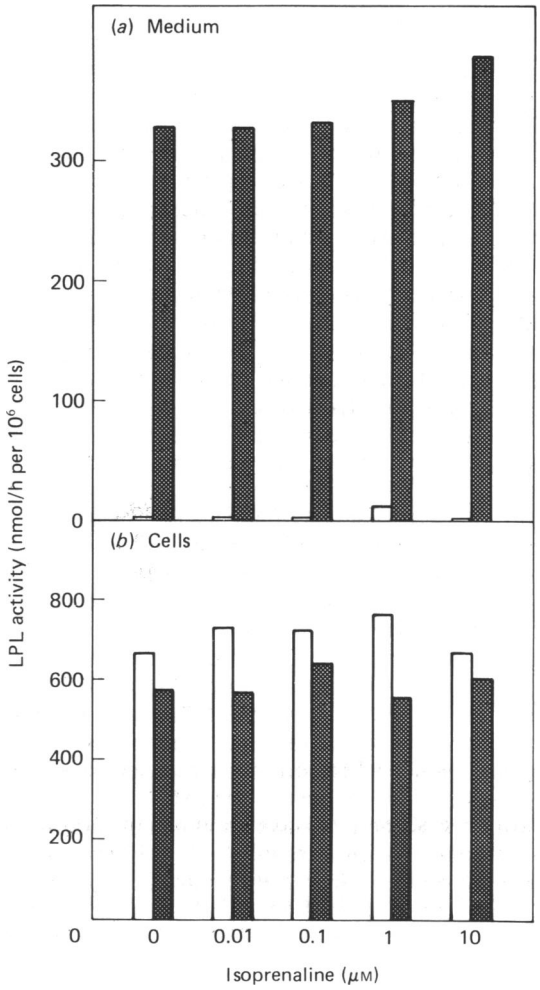

Fig. 2. Effect of incubating myocytes with isoprenaline on heparin-releasable and cellular LPL activities

Myocytes were incubated for 30 min with the indicated concentrations of isoprenaline. LPL activity in the incubation medium (*a*) and in the cell pellet after centrifugation (*b*) was then measured before (□; pre-heparin) and again 10 min after addition of 5 units of heparin/ml (■; post-heparin).

Treatment of myocytes with 10 μM-isoprenaline results in a 3–4-fold increase in cyclic AMP [16]. Forskolin activates the catalytic subunit of adenylate cyclase [18] and produces a profound increase in cyclic AMP in myocytes [16]. As shown in Fig. 3, 100 μM-forskolin resulted in a progressive increase in cyclic AMP, to values that are 40-fold greater than in control cells. Nevertheless, forskolin had no effect on the basal or heparin-induced release of LPL into the medium. Preincubation of myocytes for 30 min with 500 μM-8-(4-chlorophenylthio) cyclic AMP, a cyclic AMP analogue that can mimic the effects of isoprenaline on the metabolism of cardiac myocytes [16], also had no effect on heparin-releasable LPL activity; the activity of LPL in the medium 5 min after the addition of heparin to control and chlorophenylthio cyclic AMP-treated myocytes was 241 ± 20 and 246 ± 10 nmol/h per 10^6 cells respectively ($n = 6$).

The 30 min preincubation of myocytes with various concentrations of isoprenaline had no significant effect on LPL activity in sonicated preparations of cells collected by centrifugation either before or after the addition of heparin (Fig. 2*b*). It should be noted that the fall in cellular LPL activity after heparin was less than the heparin-induced release of LPL into the incubation medium (Fig. 2). Latency of LPL in the pre-heparin cell homogenates cannot explain this discrepancy, since treatment of the sonicated myocytes with Triton X-114 [19] did not increase enzyme activity. The lack of effect of isoprenaline on LPL activity in sonicated cells after centrifugation is also shown in Table 1, where incubation conditions (15 min in Krebs–Henseleit bicarbonate buffer) were the same as used by Palmer & Kane [15]. Isoprenaline also had no effect on LPL activity measured in acetone/ether-dried-powder preparations (Table 1). With both types of preparation, isoprenaline increased the phosphorylase *a* activity ratio.

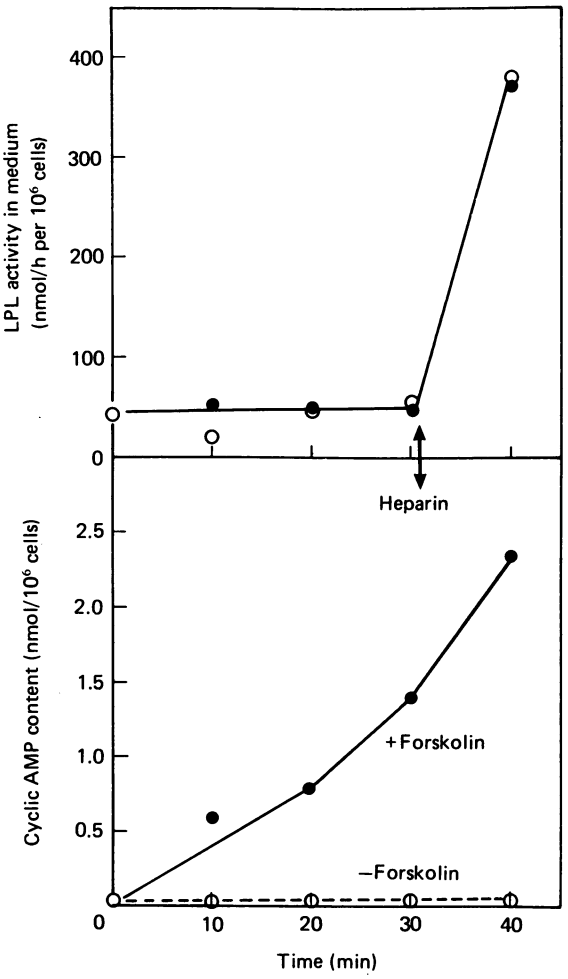

Fig. 3. Effect of incubation of myocytes with forskolin on LPL activity in the medium and on the content of cyclic AMP

Myocytes were incubated in the absence (○) and in the presence (●) of 100 μM-forskolin. At the indicated times, LPL activity in the medium and the total content of cyclic AMP in the cells plus medium were measured. Heparin (5 units/ml) was added to the incubation at the time indicated by the arrow.

DISCUSSION

Addition of heparin to cardiac myocytes resulted in the rapid release of LPL into the incubation medium. A similar rapid release of LPL induced by heparin has been observed with cultured heart mesenchymal cells [5–7], cultured mouse Ob17 preadipocytes [20,21] and fat-cells

Table 1. Effect of isoprenaline on LPL and phosphorylase activities

LPL activity, measured in the absence and in the presence of serum, and the phosphorylase *a* activity ratio were measured with the indicated preparations from myocytes incubated for 15 min in the absence and in the presence of isoprenaline (10 μM). The numbers of myocyte preparations are given in parentheses. Results are means ± S.E.M.; $*P < 0.02$, $**P < 0.01$ relative to preparations from myocytes incubated in the absence of isoprenaline.

Enzyme preparation	Isoprenaline	LPL activity (nmol/h per 10^6 cells) −Serum	LPL activity (nmol/h per 10^6 cells) +Serum	Phosphorylase activity ratio (−AMP/+AMP)
A. Sonicated cell pellet after centrifugation	−	58.7±5.9 (8)	554±96 (8)	0.25±0.03 (5)
	+	55.3±7.1 (8)	575±95 (8)	0.37±0.02 (5)*
B. Acetone/ether-dried powder	−	14.2±3.3 (10)	344±57 (10)	0.05±0.01 (11)
	+	14.9±2.7 (10)	390±57 (10)	0.15±0.02 (11)**

[22]. It is most likely that heparin can rapidly release LPL from sites that are at or near the cell surface of myocytes. A glycocalyx surrounding isolated cardiac myocytes can be observed by electron microscopy [23; D. L. Severson, unpublished work], and immunodetectable LPL has been observed at the cell surface of adipocytes [24,25]. Previous studies with heparin addition to cardiac myocytes isolated from adult rat hearts observed only a slow release of LPL measured during incubations of 1–4 h [12,13]. Differences in the myocyte isolation techniques probably accounts for this discrepancy; Al-Jafari & Cryer [25] have shown that the use of increased concentrations of collagenase during the preparation of adipocytes resulted in the loss of cell-surface LPL.

Preincubation of myocytes with isoprenaline, forskolin or 8-(4-chlorophenylthio) cyclic AMP for 30 min did not change either the basal or the heparin-induced release of LPL into the medium. Therefore myocytes are clearly different from neonatal-heart mesenchymal cells, where incubation with isoprenaline for 30 min or with dibutyryl cyclic AMP for 2 h increased heparin-releasable LPL activity [7]. Cryer *et al.* [13] reported that dibutyryl cyclic AMP had no effect on LPL secreted from myocytes incubated with heparin for 1–4 h.

Incubation with isoprenaline also had no effect on cellular LPL activity, measured with or without heparin treatment. LPL activity was measured both in sonicated preparations and in acetone/ether-dried-powder preparations, and the myocardial cells were sensitive to the catecholamine, as evidenced by an increase in the phosphorylase *a* activity ratio. Thus we cannot confirm the report of Palmer & Kane [15] that a 15 min incubation of myocytes with adrenaline could increase the serum-stimulated lipase activity that has the characteristics of LPL. The reason for this discrepancy is not apparent. With cultured heart mesenchymal cells, the isoprenaline-induced increase in heparin-releasable LPL activity was coupled to a corresponding decline in cellular activity, so that total cellular activity did not change [7].

Studies on the effect of catecholamines on residual (heparin-non-releasable) LPL activity in perfused hearts are also controversial. Palmer *et al.* [26] have reported that LPL-like activity in heart homogenates was increased by perfusion with adrenaline, phosphodiesterase inhibitors or dibutyryl cyclic AMP, but other investigators have shown that perfusion of hearts with noradrenaline or glucagon produced either no change [27] or a decline [28,29] in residual LPL activity in post-nuclear fractions from heparin-perfused hearts, depending on the time of perfusion. There is a clearer consensus, however, that a chronic increase in cyclic AMP produced by the administration of cholera toxin to intact rats does increase heart LPL activity in both heparin-releasable and residual fractions [30,31]. Therefore, the long-term exposure of myocytes to catecholamines may increase LPL activity by changing rates of glycosylation and/or synthesis, as has been reported for mesenchymal cells [6,7].

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