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Treatment of cardiac myocytes with 8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate, forskolin or cholera toxin does not stimulate cellular or heparin-releasable lipoprotein lipase activities

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Incubation of isolated cardiac myocytes with 500 μM-8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPT-cAMP) or 100 μM-forskolin for 2½ h did not increase the heparin-induced release of lipoprotein lipase (LPL) into the medium. When LPL activity in cardiac myocytes was depleted by treatment of rats with cycloheximide (2 mg/kg; 2.5 h) and inclusion of the protein-synthesis inhibitor in the isolation solutions, incubation with CPT-cAMP or forskolin did not influence the rate of repletion of LPL activity in cells or the recovery of heparin-releasable LPL activity. Although the administration of cholera toxin (0.5 mg/kg; 16–17 h) to rats increased LPL activity in a low-speed supernatant fraction from heparin-perfused hearts, LPL activity was not increased in cardiac myocytes from cholera-toxin-treated rat hearts, and the heparin-induced release of LPL was unchanged. Incubation of cultured ventricular myocytes with 1 μg of cholera toxin/ml or 500 μM-CPT-cAMP for 24 h did not increase cellular LPL activity or LPL released into the culture medium after a 40 min incubation with heparin. Therefore interventions that stimulate adenylate cyclase activity (forskolin, cholera toxin) or incubation with CPT-cAMP do not increase cellular LPL activity or promote the translocation of LPL to a heparin-releasable fraction in cardiac myocytes.

INTRODUCTION

Lipoprotein lipase (LPL) is located at the luminal surface of vascular endothelial cells and catalyses the hydrolysis of the triacylglycerol component of circulating lipoproteins [1,2]. Endothelial cells do not synthesize LPL [3], therefore the enzyme must be translocated to its functional site at the vascular endothelium after synthesis, processing and secretion from parenchymal cells. Perfusion of hearts with heparin displaces LPL from endothelial binding sites (heparin-releasable LPL activity) and decreases the ability of perfused hearts to degrade triacylglycerol-rich lipoproteins [2]; the heparin-non-releasable or residual LPL will be present in the interstitial space [4,5], cardiac myocytes [5] and perhaps other cells in the heart.

Perfusion of rat hearts with noradrenaline for 2 and 4 h resulted in an increase in heparin-releasable LPL activity and a decline in residual LPL [6], indicating that stimulation of cyclic AMP formation produced a redistribution of LPL in the heart. Administration of cholera toxin to rats for 16 h resulted in an increase in both heparin-releasable and residual LPL activities in the heart [7–9]. Therefore the chronic elevation of cyclic AMP may increase both the synthesis and translocation of LPL.

LPL is present in cultured mesenchymal cells from neonatal rat hearts, and heparin induces the release of LPL into the medium [10,11]. Incubation of cultured mesenchymal cells with isoprenaline or dibutyryl cyclic AMP for 1–2 h produced an increase in heparin-releasable LPL activity, with a concomitant decrease in residual activity [11]. Increasing the time of incubation with isoprenaline [11] or dibutyryl cyclic AMP [10] to 24 h resulted in an increase in both heparin-releasable and residual LPL activities. Therefore the isoprenaline-induced formation of cyclic AMP stimulated both the secretion and the synthesis of LPL in cardiac mesenchymal cells.

In the adult rat heart, cardiac myocytes are the principal, if not the exclusive, source of LPL [5,12] that is the precursor of the functional endothelium-bound enzyme. The heparin-induced release of LPL into the incubation medium of cardiac myocytes [13–15] is characterized by two phases: a rapid release (5–10 min) from sites that are presumably at or near the myocardial cell surface [14,15], followed by a slower rate of release (10–120 min) that is dependent on protein synthesis and microtubular function [13–15]. A short-term preincubation (30 min) of cardiac myocytes with isoprenaline or forskolin did not change cellular or heparin-releasable LPL activities measured after a 5–10 min incubation with heparin [16]. The objective of the present investigation was to determine if the slow protein-synthesis-dependent phase of LPL secretion in response to heparin could be influenced by incubation of cardiac myocytes with forskolin or a cyclic AMP analogue, 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPT-cAMP). In addition, cellular LPL activity and heparin-induced LPL release were determined with cardiac myocytes isolated from rats after administration of cholera toxin *in vivo*, and compared with results on cultured myocytes incubated with cholera toxin or CPT-cAMP.

EXPERIMENTAL

Male Sprague–Dawley rats (200–300 g) were fed on a laboratory chow diet (Wayne Rodent Blox) *ad libitum*, and were maintained on a light (07:00–19:00 h)/dark cycle. Rats were killed from 10:30 to 11:00 h. Ca^{2+}-tolerant myocytes were isolated essentially as described by Kryski *et al.*, [17], except that the collagenase concentration was decreased to 156 units/ml. The viability of myocytes, assessed as the percentage of rod-shaped cells that excluded Trypan Blue, was 75–80%. Myocytes were finally suspended in Joklik minimal essential medium containing 1.5 mM-$CaCl_2$ and 1% (w/v) defatted BSA [17] to a cell density

Abbreviations used: LPL, lipoprotein lipase; CPT-cAMP, 8-(4-chlorophenylthio) cyclic AMP.

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of 4×10^5 cells/ml, and were incubated at 37 °C under O_2/CO_2 (19:1). The release of LPL into the incubation medium was measured as described previously [14–16]. Heparin was added to the myocyte incubation medium to give a final concentration of 5 units/ml, and at various times 1 ml samples were removed and centrifuged for 10 s at 15000 *g* in an Eppendorf centrifuge. The supernatant (medium) was removed, frozen and stored at −80 °C until assayed for LPL activity. Cell pellets after centrifugation were also frozen, and then homogenized by sonication for LPL activity determinations.

LPL activity in the medium was measured with a sonicated [^{3}H]triolein substrate emulsion [14,18] and is expressed as nmol of oleate released/h per 10^6 cells. The standard assay conditions were: 0.6 mM-glycerol tri[9,10(n)-^{3}H]oleate (1 mCi/mmol), 25 mM-Pipes, pH 7.5, 0.05% BSA, 50 mM-$MgCl_2$, 2% (v/v) chicken serum and usually 100 μl of medium in a total volume of 400 μl. When LPL activity was measured in sonicated cells, 1.25 units of heparin/ml were included in the assay so that assay conditions were the same as with post-heparin medium. All LPL assays of the medium or sonicated cells were performed in duplicate under conditions where activity was linear with respect to time and protein.

In some experiments as noted in the text, cycloheximide was used to deplete LPL in cardiac myocytes [19]. Rats were injected (intraperitoneally) with cycloheximide (2 mg/kg) or saline 2.5 h before death and removal of the hearts. Cardiac myocytes were then isolated as described above [17], except that 50 μM-cycloheximide was included in the isolation solutions. Myocyte incubations were then performed as usual in the absence of cycloheximide.

In order to investigate the effects of cholera toxin *in vivo*, rats were anaesthetized with sodium pentobarbital (40 mg, intraperitoneally) and then injected with cholera toxin (0.5 mg/kg, intravenously) as described by Knobler *et al.* [7]. Control rats were injected with saline. Rats were killed 16–17 h later; hearts were removed and used for the isolation of cardiac myocytes. In addition, LPL activity was determined after perfusion of control or cholera-toxin-treated hearts for 5 min with Joklik minimal essential medium containing 1.5 mM-$CaCl_2$ and 5 units of heparin/ml to remove endothelium-bound LPL. Ventricles were minced and homogenized in 15 ml of a sucrose buffer (0.25 M-sucrose/1 mM-EDTA/1 mM-dithiothreitol/10 mM-Hepes, pH 7.5) with a Polytron tissue disintegrator (2 × 30 s). The homogenate was centrifuged at 1000 *g* for 15 min, and LPL activity (nmol of oleate released/h per mg of protein) was measured in the low-speed supernatant fraction.

Ventricular myocytes were also maintained in serum-free culture [20]. To decrease bacterial and fungal contamination, all cell isolation and plating procedures were performed in laminar flow cabinets, and all solutions were sterilized by filtration (0.22 μm pore size). Hearts from adult male rats were first perfused with a non-recirculating Ca^{2+}-free Krebs–Ringer bicarbonate (KRB) buffer, consisting of (mM) 110 NaCl, 2.6 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 11 glucose (pH maintained at 7.4 with O_2/CO_2, 19:1), for about 4 min at 10 ml/min to remove blood from the coronary circulation, followed by perfusion for 20–25 min with recirculating KRB containing 25 μM-$CaCl_2$ and collagenase (40–50 units/ml). The flaccid heart was removed from the cannula, and two-thirds of the ventricle was minced and incubated with 20 ml of the recirculation collagenase solution for 5–10 min. The minced tissue was gently titurated 2–3 times/min with a 3.5 mm-diameter pipette to release the myocytes. The cell suspension was filtered through nylon gauze (183 μm pore size), centrifuged for 3 min at 34 *g* and successively resuspended in KRB containing 0.2 mM-, 0.5 mM- and 1 mM-$CaCl_2$. The myocytes were then centrifuged a fourth time (3 min at 34 *g*) and resuspended in culture medium M199 supplemented with 100 μg of streptomycin/ml and 100 units of penicillin/ml. Myocytes were counted in a Sedgewick Rafter chamber, and plated in 35 mm-diameter culture dishes (Falcon 1008) containing 1.8 cm × 1.8 cm HCl-etched glass coverslips at a density of 6.2×10^4 cells/cm^2. This resulted in about 2×10^4 myocytes per coverslip; the coverslips had been preincubated for 1 h at 37 °C or 25 °C with 12.5 μg of laminin/cm^2. Cultured myocytes were stored at 37 °C in a humidified atmosphere of O_2/CO_2 (19:1). The culture medium was changed after 1 h to remove unattached cells and debris, and replaced with 3 ml of fresh M199; approx. 80–85% of the myocytes had attached during this initial 1 h incubation.

Cholera toxin (1 μg/ml) or CPT-cAMP (500 μM) was added to the fresh M199 in duplicate culture dishes, and these treated myocyte cultures, together with duplicate control cultures, were incubated for 24 h. The culture medium was then removed and replaced with 1 ml of Joklik minimal essential medium containing 1.5 mM-$CaCl_2$ and 1% defatted BSA. The number of attached cells was determined by counting three random fields of 1.59 cm^2 per coverslip in a phase-contrast microscope. In order to measure heparin-releasable LPL activity, heparin (5 units/ml) was added to one of the culture dishes, and the incubation was continued for a further 40 min; the second culture dish was incubated in the absence of heparin. Myocytes were then scraped from the coverslips with a rubber policeman, and the resuspended myocytes were centrifuged for 10 s at 15000 *g* in an Eppendorf micro-centrifuge. The medium was removed, and cell pellets were washed twice with sucrose buffer to remove BSA in the Joklik medium. The cell pellets were homogenized by sonication, and LPL activity in the sonicated cells and medium was determined as described above, except that the [^{3}H]triolein concentration was decreased to 0.1 mM so that the specific radioactivity of the substrate was increased to 6 mCi/mmol, incubation temperature was increased from 30 °C to 37 °C and the standard assay incubation time was increased from 30 min to 60 min in order to increase the sensitivity of the assay. LPL activity was calculated either as nmol of oleate released/h per 10^6 cells (based on the cell count after culture) or as nmol of oleate released/h per mg of protein in the sonicated cells. For determination of cyclic AMP content, $HClO_4$ (final concn. 5%, v/v) was added to the cultured myocytes in 1 ml of Joklik medium. After centrifugation, the deproteinized supernatant was neutralized and cyclic AMP was measured by radioassay [16,17].

Protein content of the low-speed supernatant fraction of perfused hearts and cultured sonicated cells was determined with a Coomassie Blue spectrophotometric assay [21].

Materials were from the sources described previously [14–16], except for the following. Cycloheximide, cholera toxin and forskolin were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). CPT-cAMP was obtained from Boehringer-Mannheim (Dorval, Que., Canada), laminin was from Collaborative Research (Bedford, MA, U.S.A.), and Joklik and M199 media were from Gibco (Burlington, Ont., Canada). Collagenase used for the isolation of cardiac myocytes for culture was purchased from Yakult (Tokyo, Japan).

RESULTS AND DISCUSSION

Previously we reported that a 30 min preincubation of cardiac myocytes with isoprenaline or forskolin had no effect on LPL activity released into the medium after a 5–10 min incubation with heparin [16]. As shown in Fig. 1, 500 μM-CPT-cAMP, a cyclic AMP analogue that can mimic the effects of isoprenaline on the metabolism of cardiac myocytes [17], had no effect on the basal rate of LPL secretion or on the heparin-induced rate of release measured for 120 min. At the end of the incubation (150

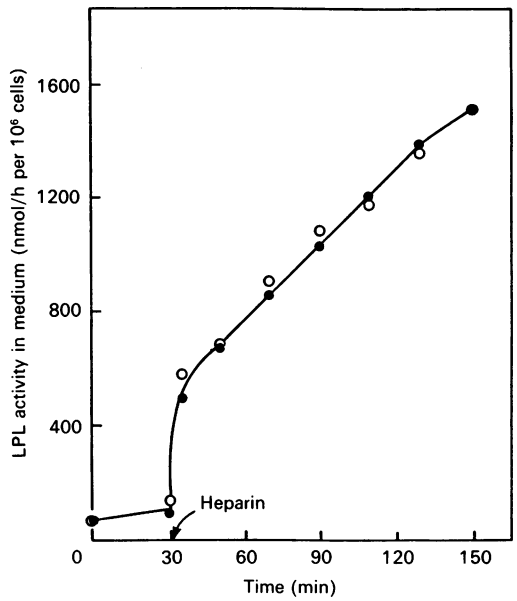

Fig. 1. Effect of CPT-cAMP on the heparin-induced release of LPL from cardiac myocytes

Myocytes were incubated in the absence (○) and in the presence (●) of 500 μM-CPT-cAMP; heparin (5 units/ml) was added after 30 min of incubation. At the indicated times, LPL activity in the medium was determined.

min), LPL activity determined in control and CPT-cAMP-treated sonicated cell preparations was 1930 and 1790 nmol/h per 10^6 cells respectively. The inability of CPT-cAMP to influence the activity of LPL released into the medium of cardiac myocytes after only a 10 min incubation with heparin was reported previously [16]. Cryer *et al.* [13] also observed that 1 mM-dibutyryl cyclic AMP did not stimulate the heparin-induced release of LPL from cardiac myocytes.

Forskolin produces a much greater increment of the cyclic AMP content of cardiac myocytes than does isoprenaline [16,17]. After incubation of cardiac myocytes with 100 μM-forskolin for 120 min, heparin-released LPL activity in the medium was 1175 nmol/h per 10^6 cells for control incubations and 925 nmol/h per 10^6 cells for myocytes incubated with forskolin. Thus activation of adenylate cyclase by forskolin or treatment with CPT-cAMP had no stimulatory effect on the heparin-induced release of LPL from cardiac myocytes during 2 h incubations, as had been observed previously during 5–10 min incubations with heparin [16]. These results are in marked contrast with experiments with cultured mesenchymal cells from neonatal-rat hearts, where a 1–2 h incubation with isoprenaline or dibutyryl cyclic AMP increased heparin-releasable LPL activity with a corresponding decrease in cellular enzyme activity, so that total LPL activity did not change [10,11]. Stam & Hulsmann [6] reported that perfusion of rat hearts with noradrenaline for 2–4 h increased heparin-releasable LPL activity from the vascular endothelium, which was accompanied by a fall in residual enzyme activity. It is unlikely that this change in LPL activity in whole perfused hearts is due to an effect of the catecholamine on LPL activity in non-myocytic cells derived from mesenchymal cells, since cardiac myocytes represent the principal, if not the exclusive, source of LPL in the adult rat heart [5,12] for translocation through the interstitial space to the vascular endothelium. The translocation of LPL observed in perfused hearts in response to β-adrenergic stimulation may not be a direct effect on cardiac myocytes, but may instead be a secondary response to the inotropic and chronotropic effects of the catecholamine, since Simpson [22] observed that the isoprenaline-induced release of LPL was inhibited in K^+-arrested perfused hearts.

Since CPT-cAMP and forskolin had no effect on the slow protein-synthesis-dependent phase of LPL secretion in response to heparin with normal cardiac myocytes (Fig. 1), it was decided to test these agents on cardiac myocytes after cycloheximide treatment had decreased cellular LPL activity [19], in order to determine if there was an effect on the repletion of LPL activity which would involve new enzyme synthesis and processing (glycosylation), followed by microtubular transport of LPL to the cell surface for release by heparin. Cyclic AMP regulates gene expression in mammalian cells [23], and an elevation in cyclic AMP contents of hearts by a 2 h perfusion with glucagon, forskolin or the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine increased rates of protein synthesis [24]. A stimulation of glycosylation has been observed after a 2 h incubation of cultured mesenchymal cells [11] and rat parotid cells [25] with isoprenaline.

Administration of cycloheximide to rats before death and inclusion of the protein-synthesis inhibitor in the isolation solutions resulted in a decrease in the initial cellular LPL activity to 12% of control (Fig. 2). Addition of cycloheximide to the incubation medium also prevented the heparin-induced release of LPL. At the end of the 60 min incubation with heparin, LPL activity in the control and cycloheximide-treated myocytes was 1000 and 150 nmol/h per 10^6 cells respectively, indicating that the continued presence of cycloheximide during the incubation of myocytes prevented any resynthesis and repletion of LPL activity in the cells, and thus no LPL activity could be released into the medium by heparin (Fig. 2). When the experimental protocol was modified to shorten the treatment of rats with cycloheximide *in vivo* to 2.5 h, the initial cellular LPL activity was decreased to 120 ± 15 nmol/h per 10^6 cells ($n = 5$). Incubation of these cells in the absence of cycloheximide resulted in a gradual increase in cellular LPL activity, which was followed by a recovery of the heparin-induced release of LPL into the medium; results from a typical experiment are shown in Fig. 3. The

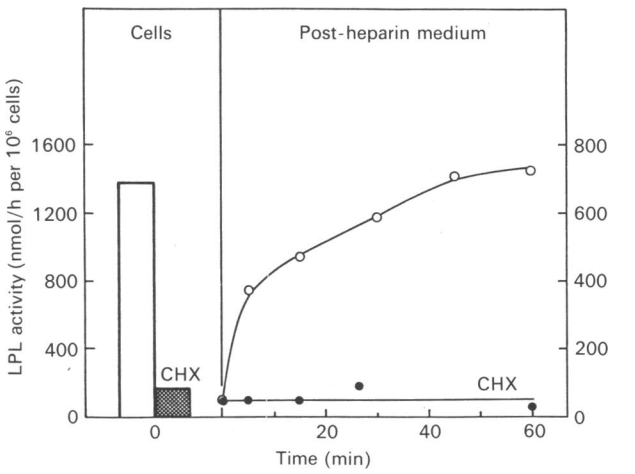

Fig. 2. Effect of cycloheximide (CHX) on cellular and heparin-releasable LPL activities

Myocytes were isolated 4 h after the intraperitoneal injection of rats with either CHX (2 mg/kg) or saline; CHX (6.6 μM) was also included in both the isolation and incubation solutions. LPL activity was determined in cell sonicates from control (□) and CHX-treated rats (■). Heparin was then added to the incubation, and the release of LPL into the medium of control (○) and CHX-treated myocytes (●) was determined at the indicated times.

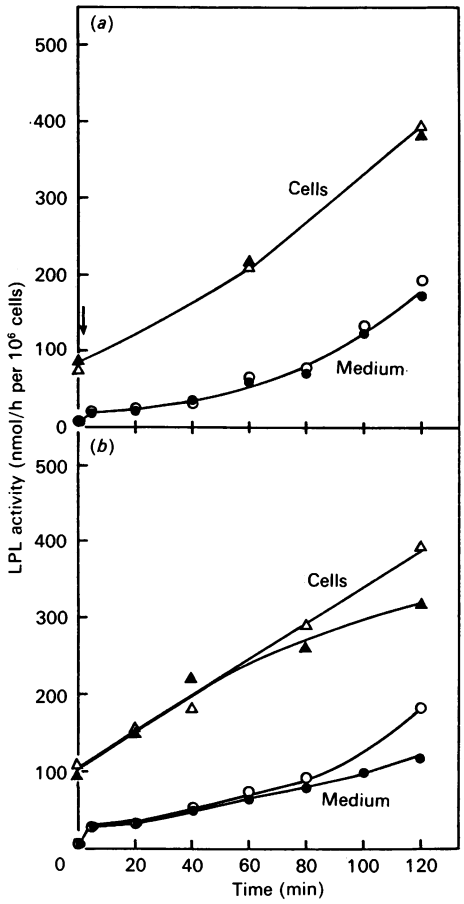

Fig. 3. Effect of CPT-cAMP and forskolin on control and heparin-releasable LPL activities in cycloheximide-treated myocytes

Myocytes were isolated after cycloheximide treatment as described in the Experimental section. (*a*) Myocytes were incubated in the absence (○, △) and in the presence (●, ▲) of 500 μM-CPT-cAMP; heparin was added at the time indicated by the arrow. LPL activity was determined in cell pellets (△, ▲) and in the medium (○, ●) at the indicated times of incubation. (*b*) As described above, except that myocytes were incubated in the absence (○, △) and in the presence (●, ▲) of 100 μM-forskolin. Similar results were obtained in two additional experiments.

Table 1. Effect of administration of cholera toxin *in vivo* on LPL activities in perfused hearts and isolated cardiac myocytes

LPL activities were determined in low-speed supernatant fractions from heparin-perfused hearts and in isolated cardiac myocytes from control and cholera-toxin-treated rats as described in the Experimental section. Results are presented as means ± S.E.M. ($n = 3$).

	LPL activity	
Treatment	Hearts (nmol/h per mg of protein)	Myocytes (nmol/h per 10^6 cells)
Control	711 ± 23	1080 ± 70
Cholera toxin	1720 ± 268	998 ± 74

presence of 500 μM-CPT-cAMP (Fig. 3*a*) or 100 μM-forskolin (Fig. 3*b*) did not increase either the rate of repletion of cellular LPL activity or the recovery of heparin-induced release of LPL into the medium. In fact, after 120 min of incubation, the heparin-releasable LPL activity from forskolin-treated myocytes was 54 ± 6 % (mean ± S.E.M., $n = 3$) of LPL activity released from control myocytes. The release of LPL in response to heparin was also slightly decreased (78 % of control; $n = 2$) after 120 min of incubation with 10 μM-forskolin. Therefore, forskolin and CPT-cAMP did not stimulate any of the intracellular processes (synthesis, processing by glycosylation, microtubular transport) that account for repletion of cellular LPL activity after cycloheximide treatment and the release of LPL by heparin in this 2 h time interval. Therefore, experiments were designed to study the effect of a more chronic elevation of cyclic AMP in cardiac myocytes.

Treatment of rats with cholera toxin for 16 h increased residual LPL activity in heparin-perfused hearts [7–9]. Similar results are shown in Table 1, where the administration of cholera toxin *in vivo* produced a 2.4 ± 0.3-fold increase in LPL activity in a low-speed supernatant fraction from perfused hearts. The increase in lipase activity in cholera-toxin-treated hearts had the characteristics of LPL, since activity was decreased by 85–95 % either by omission of serum from the assay or by increasing the ionic strength to 1 M. However, no effect of cholera-toxin treatment on LPL activity was observed after isolation of cardiac myocytes (Table 1). Furthermore, the heparin-induced release of LPL from cardiac myocytes prepared from cholera-toxin-treated rats was identical with the rate from control myocytes (Fig. 4). It could be argued that the cholera-toxin-induced increment in residual LPL activity in preparations from whole perfused hearts was originally in myocardial cells, but was inactivated during the isolation procedures involving treatment with collagenase [26]. Consequently, the effects of cholera toxin *in vitro* were determined with cultured myocytes.

Incubation of cultured mesenchymal cells with dibutyryl cyclic AMP [10] or isoprenaline [11] for 24 h increased both heparin-releasable and cellular LPL activities, so that total enzyme activity was elevated. Furthermore, the incorporation of radiolabelled leucine into immunoadsorbable LPL was increased after a 24 h incubation of cultured mesenchymal cells with isoprenaline [11]. In contrast, incubation of cultured myocytes for 24 h with either cholera toxin or CPT-cAMP had no effect on cellular LPL activity (Table 2); LPL activity in cholera-toxin-treated cells (incubated in the absence of heparin) was 100 ± 7 % ($n = 4$) of the activity in control cardiac myocytes. Incubation of the cultured myocytes with cholera toxin or CPT-cAMP also did not increase LPL activity released into the culture medium after

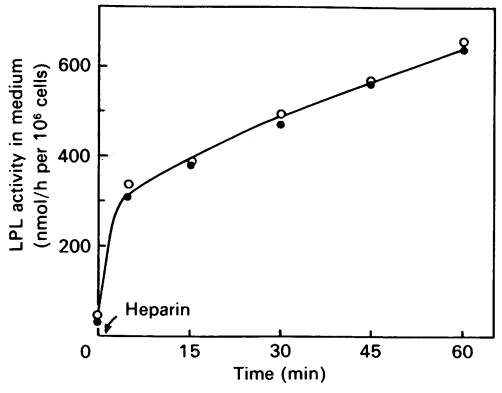

Fig. 4. Effect of the administration of cholera toxin *in vivo* on the heparin-induced release of LPL from cardiac myocytes

Myocytes were isolated from control (○) and cholera-toxin-treated rats (●). At the time indicated by the arrow, heparin (5 units/ml) was added to the incubation, and LPL activity in the medium was determined at the indicated times. Results are the mean of two experiments.

Table 2. Effect of cholera toxin and CPT-cAMP on LPL activity in cultured cardiac myocytes

Cardiac myocytes were cultured (duplicate dishes) for 24 h in medium containing no additions (control), 1 μg of cholera toxin/ml or 500 μM-CPT-cAMP. The culture medium was changed, and a single culture dish was incubated for an additional 40 min in the presence of heparin (5 units/ml); the second culture dish was incubated in the absence of heparin. LPL activity was then determined in cells and medium. Results are from a single representative experiment; similar results were obtained in two or three additional experiments.

Preincubation additions	LPL activity (nmol/h per mg of protein)			
	Cells		Medium	
	−Heparin	+Heparin	−Heparin	+Heparin
None	244	217	5.6	32.5
Cholera toxin (1 μg/ml)	243	224	5.0	26.9
CPT-cAMP (500 μM)	241	227	3.0	23.0

a 40 min incubation with heparin (Table 2). For example, the net increment in LPL activity in the medium caused by incubation with heparin was 13.1 ± 4.8 ($n = 4$) and 15.5 ± 3.7 ($n = 3$) nmol/h per mg for control and CPT-cAMP-treated culture dishes respectively. The cyclic AMP content of cultured myocytes was increased by 2.6-fold ($n = 2$) after 5 h of incubation with cholera toxin. This increase in cyclic AMP content, together with the observation that residual LPL activity was increased in perfused hearts (Table 1), as expected, indicated that the cholera-toxin preparation was biologically active. The inability to observe a direct effect of cholera toxin *in vitro* with cultured myocytes (Table 2) would argue against the suggestion that the cholera-toxin-induced increment in LPL activity observed in heparin-perfused hearts was selectively inactivated from, for example, the surface of cardiac myocytes by collagenase during the isolation procedure. Rather, the increase in residual LPL seen in cholera-toxin-treated hearts must be present in a non-myocytic compartment, i.e. some other cell types(s), or in the interstitial space [4,5]. An increase in interstitial LPL would be consistent with the observation that cholera-toxin treatment also increases the heparin-releasable fraction of LPL present at the endothelial cell surface of the coronary circulation [7–9]. Furthermore, any interstitial LPL would be readily lost during the cardiac myocyte isolation procedures. Fasting of rats for 24 h also produced an increase in heparin-releasable LPL activity from the vascular endothelium [27] and in interstitial LPL activity [4], with no change in LPL activity in isolated cardiac myocytes ([12]; R. Carroll & D. L. Severson, unpublished work). Since cholera-toxin treatment *in vitro* had no direct effect on cellular or heparin-releasable LPL activities in cultured cardiac myocytes (Table 2), another possibility to be considered is that the administration of cholera toxin *in vivo* may act on some non-parenchymal cell(s) to produce some signal so that the production and secretion of LPL is stimulated in cardiac myocytes. In the liver, eicosanoids are involved in intercellular communication; prostaglandin D_2 produced by non-parenchymal cells (Kupffer and endothelial cells) in response to a variety of stimuli induced glycogenolysis in parenchymal cells [28].

In summary, treatment of cardiac myocytes from adult rats with CPT-cAMP, forskolin and cholera toxin for either 2 or 24 h does not increase either cellular or heparin-releasable LPL activities, consistent with previous findings [13,16]. LPL activity in the post-heparin medium of cardiac myocytes was not increased by preincubation with the catalytic subunit of protein kinase A [29]. Therefore, an elevation in cyclic AMP content must not directly regulate cellular LPL activity or the heparin-induced release of LPL from cardiac myocytes, in contrast with results obtained with cultured mesenchymal cells from neonatal-rat hearts [10,11]. Further investigations are required to determine the precise compartments in perfused hearts that contain the increased LPL activity after administration of cholera toxin *in vivo*.

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