Hormone-stimulated lipolysis in cardiac myocytes

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Type L hormone-sensitive lipase (HSL) activity was increased approx. 35% above control in cardiac myocytes incubated for 15 min with 5 nM-adrenaline. Concomitantly, adrenaline-stimulated myocytes had a lower triacylglycerol content, released more non-esterified fatty acid and had a higher intracellular concentration of cyclic AMP than did myocytes incubated without hormone. The lipase activity measured in adrenaline-stimulated and non-stimulated myocytes was stable in acetone/diethyl ether, stimulated by serum and inhibited by NaCl. These properties are consistent with the type L designation of this HSL. The finding that type L HSL is stimulated by adrenaline indicates that the enzyme that is being activated is found in the cell and not associated with an extracellular compartment of the myocardium.

Injection of glucagon into the rat produced a reduction in the concentration of cardiac TG (Oscai, 1979). Concomitantly, there was an elevation of the activity of a TG lipase measured in heart homogenates. Perfusion of rat hearts with adrenaline produces a similar response (Palmer *et al.*, 1981). The TG concentration was reduced and the activity of a lipase with properties similar to those described for LPL was increased in hormone-perfused hearts. Since hormone sensitivity has been demonstrated, and because no plasma lipoproteins exist within heart parenchymal cells, this enzyme has been called type L (having the properties of LPL) HSL (Palmer & Kane, 1983).

From these previous experiments, it has been postulated that enzymic activity measured in heart homogenates was intracellular because hearts were perfused with heparin before the experimental treatment to remove LPL from the capillary beds. However, it has been postulated that a pool of LPL also exists in the interstitial spaces between cardiac cells (Jansen *et al.*, 1980). Therefore, it was the purpose of the present study to determine if type L HSL activity could be stimulated by adrenaline in preparations of isolated cardiac myocytes. In this preparation, changes in enzyme activity would be cell-associated and not related to activity changes in enzyme located in extracellular compartments.

Abbreviations used: TG, triacylglycerol; LPL, lipoprotein lipase; HSL, hormone-sensitive lipase.

Methods

Animal care

Male rats of the Wistar strain weighing about 90 g were purchased from Charles River Breeders (Wilmington, MA, U.S.A.). Animals were provided unrestricted access to a diet of Purina chow and water. The animal room was maintained at a temperature between 21 and 23°C and lighted between 07:00 and 19:00 h. The rats were permitted a minimum 3 weeks to adapt to the living conditions in our animal facilities. Fed rats weighing approx. 250 g were killed between 08:00 and 09:00 h to negate circadian rhythms in lipase activity. The rats were anaesthetized with sodium pentobarbital (50 mg/kg body wt.) and killed by removing the heart.

Isolation of myocytes

Cardiac myocytes were isolated by a modification of the technique described by Powell & Twist (1976). Hearts were perfused with calcium-free Krebs bicarbonate buffer containing 11.1 mM-glucose (KBG), in a flow-through system until perfusates were clear of blood. Each heart was perfused for 20 min more in a recirculating system with KBG buffer containing 0.75 mg of collagenase/ml. After perfusion, hearts were trimmed free of atria, great vessels and other adhering tissue, then minced in KBG buffer containing 2% fatty acid-free bovine serum albumin and 0.75 mg of collagenase/ml in a total volume of 10 ml/heart. The tissue suspension was transferred to a flask and shaken at

Table 1. *Lipolytic responses of rat heart myocytes to 15 min incubation with and without adrenaline*

All values are means $\pm$ S.E.M.; numbers of experiments are given in parentheses. Each experiment represents results from a separate cell preparation. HSL activity was measured in acetone/diethyl ether powders of myocytes; 1 unit = 1 μ mol of non-esterified fatty acid released/h.

Treatment	Medium non-esterified fatty acids (μ mol/g of cells)	Cellular triacylglycerol (μ mol/g of cells)	Type L HSL activity (units/g of cell powder)	Myocyte cyclic AMP (nmol/g dry weight)
Control	1.57 $\pm$ 0.13 (10)	1.84 $\pm$ 0.15 (8)	363 $\pm$ 9 (8)	0.704 $\pm$ 0.06 (5)
Adrenaline	2.17* $\pm$ 0.12 (9)	1.33* $\pm$ 0.14 (9)	481* $\pm$ 29 (7)	1.49* $\pm$ 0.19 (5)

* Significantly different from control ($P < 0.05$).

100–110 cycles/min at 37°C for 15 min. Cells were gently filtered through a nylon mesh. Non-filtered material was re-incubated for an additional 10 min to increase cell yield. The filtrate was centrifuged at 50 g for 90 s without the use of a bovine serum albumin cushion. The cells were then washed twice with KBG buffer. Tissue and cells were gassed continuously throughout the entire preparation with a mixture of O₂/CO₂ (19:1). This procedure consistently gives a 60–70% cell yield with a viability, as determined by Trypan Blue staining, of 75%. The viability remained at this level throughout the 15 min incubation period. Microscopic evaluation indicated that cells were primarily rod-shaped and preparations were essentially free of connective tissue and tissue debris. Less than 10% of the cells were beating spontaneously.

Cell incubation

Cells were resuspended in Krebs buffer containing 10% calf serum and no glucose. Myocytes were incubated for 15 min at 37°C with buffer alone or with buffer containing 5 nM-adrenaline. At the end of the incubation, myocytes were pelleted by centrifugation at 90 g. Incubation medium was removed from the resulting cell pellet and analysed for non-esterified fatty acid content by the method of Trout *et al.* (1960). Approx. one-half of the cell pellet was frozen and subsequently assayed for TG content by the method of Fletcher (1968) after extraction by the procedure described by Entenman (1957). The remaining half of the cell preparation was analysed for type L HSL activity after lipid extraction using acetone/diethyl ether (Palmer & Kane, 1983). Cells were homogenized in 50 vol. of cold acetone. The homogenate was then filtered and the filter was subsequently washed with acetone and diethyl ether and then freeze-dried. Defatted heart powders were then assayed for type L HSL activity by the technique described by Borensztajn *et al.* (1972), classically used to measure LPL activity. The concentration of cyclic AMP was measured in freeze-dried heart powders by the method of Brostrom & Kon (1974) after protein was removed.

Results

At the beginning of the incubation period (zero min), the cellular concentration of TG, medium content of non-esterified fatty acid and activity of myocyte type L HSL were 1.89 $\pm$ 0.09 μ mol/g of cells, 1.00 $\pm$ 0.09 μ mol/g of cells and 323 $\pm$ 11 units/g of powder respectively. It is evident from the data in Table 1 that when cardiac myocytes are incubated with buffer containing 5 nM-adrenaline for 15 min there was 37% more non-esterified fatty acid in the incubation medium than was measured in the medium of control cells incubated without hormone. Concomitantly, the concentration of TG was 28% lower than control in the hormone-stimulated cells. Type L HSL activity, measured in acetone/diethyl ether powders of myocytes incubated without hormone, was 363 $\pm$ 9 μ mol of non-esterified fatty acid released/h per g of powder. Adrenaline significantly ($P < 0.05$) increased lipase activity by 33% to 481 $\pm$ 29 μ mol of fatty acid released/h per g of heart powder. As expected, adrenaline stimulation produced a significant increase in the level of myocyte cyclic AMP.

Table 2 presents data obtained from a second set of experiments performed to study properties of type L HSL from control and adrenaline-stimulated cardiac myocytes. Incubation of myocytes with 5 nM-adrenaline stimulated type L HSL activity by 44%. When 1 M-NaCl was present in the assay, type L HSL activity from control myocytes was inhibited

Table 2. *The influence of serum and NaCl on type L HSL measured in acetone/diethyl ether powders of rat heart myocytes incubated with and without adrenaline*

All values are means $\pm$ S.E.M. for the numbers of experiments given in parentheses; 1 unit of activity = 1 μ mol of non-esterified fatty acid released/h.

	Type L HSL activity (units/g of powder)		
	Normal assay	–Serum	+ NaCl (1 M)
Control (n = 5)	316 $\pm$ 20	26 $\pm$ 3	63 $\pm$ 6
Adrenaline (n = 4) (5 nM)	456 $\pm$ 17*	33 $\pm$ 8	49 $\pm$ 10

* Significantly different from control ($P < 0.05$).

80%, whereas activity measured in acetone/diethyl ether powders prepared from adrenaline-stimulated myocytes was reduced by 89%. When serum was removed from the assay, type L HSL activity was reduced more than 90% in both the control and hormone-stimulated preparations.

Discussion

Lipolysis is stimulated in isolated cardiac myocytes when cells are incubated for 15 min with 5 nM-adrenaline. This is characterized by a reduction in the cellular content of TG and an increase in the amount of non-esterified fatty acid released into the incubation medium. The decrease in TG is not mirrored by a stoichiometric increase in medium non-esterified fatty acid concentration. This is not unusual since non-esterified fatty acid is a primary fuel for the myocardium. These data indicate that the decrease in TG reported for the adrenaline-stimulated perfused hearts (Palmer *et al.*, 1981) is associated with TG stored within the heart cells and not TG found in compartments outside the cardiocyte.

The catecholamine also significantly ($P < 0.05$) increased the activity of type L HSL. These findings contradict those of Cryer *et al.* (1981), who reported that dibutyl-AMP and glucagon had no effect on cardiac myocyte LPL activity. The difference in results could, however, be explained by comparing experimental protocols. Incubation medium contents and incubation times differed greatly in the two sets of experiments. These results not only confirm previous findings (Palmer *et al.*, 1981; Palmer & Kane, 1983) that adrenaline stimulates a cardiac TG lipase, but localize the enzymic activity to the cellular fraction of the

myocardium and not to extracellular interstitial compartments or to non-cardiac cell types. The finding that the stimulated and control enzyme activities were stable to treatment with acetone/diethyl ether, stimulated by serum and inhibited by NaCl are in agreement with the report by Vahouny *et al.* (1980), who reported similar characteristics for cardiocyte LPL. Therefore, these data support the type L designation of this cardiac HSL.

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