Relationship between type L hormone-sensitive lipase activity and endogenous triacylglycerol in the hearts of colchicine-treated rats

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Colchicine injection was used as a tool to potentiate the increase in intracellular lipoprotein lipase (type L hormone-sensitive lipase) activity normally seen with fasting to determine if elevation of enzyme activity by this method produced a reduction in endogenous triacylglycerol (TG) in rat heart. Both fasting and fasting + colchicine treatment increased total lipoprotein lipase (LPL) activity from a control value of 80 units/g to approx. 144 units/g. The initial control value was obtained at 08:00 h after overnight feeding and the final values were obtained at 17:00 h, after 9 h of fasting. Fasting alone increased activity in both the capillary-bound LPL and type L hormone-sensitive lipase (HSL) fractions of cardiac muscle. In contrast, colchicine treatment, by blocking the export of enzyme from the cell as a result of microtubular disruption, restricted the increase in enzyme activity to the intracellular fraction of the heart. There was a highly significant ($P < 0.001$) negative relationship ($r = -0.73$) between type L HSL activity and TG content in hearts of fasting and fasting + colchicine-treated rats. At a time when type L HSL activity was increased and TG content decreased, the cyclic AMP concentration of heart remained unchanged, ruling out the possibility that cyclic AMP might be activating any one of the identified cardiac TG lipases. These data provided indirect evidence that type L HSL is 'seeing the intracellular TG droplet' and that this enzyme may play a role in the regulation of myocardial lipolysis.

The heart contains two distinct pools of LPL activity (Robinson, 1970; Borensztajn, 1979). One fraction, which is readily released from heart by perfusion with heparin, is localized on the surface of endothelial cells of capillaries where it is directly involved in the hydrolysis of plasma lipoprotein TG. The second fraction, which remains in tissue after perfusion with heparin, is thought to represent a precursor or an intracellular storage pool for the endothelium-bound LPL. In contrast with this notion, over the past 8 years an attempt has been made to identify a function for the intracellular LPL fraction as a mobilizer of endogenous cardiac TG. In this type of work, glucagon (Oscai, 1979), adrenaline, cyclic AMP, and protein kinase have been shown to activate intracellular LPL and decrease TG stores (Palmer *et al.*, 1981a; Palmer & Kane, 1983a,b). Because hormone sensitivity has been demonstrated and because no plasma lipo-

proteins exist in heart parenchymal cells, this intracellular enzyme has been called type L (having the properties of LPL) HSL. In separate experiments, an inverse relationship between type L HSL activity and TG content of the heart has been observed under normal physiological conditions such as exercise (Oscai *et al.*, 1982), fasting, cold exposure, and fat feeding (Miller & Oscai, 1984). Although a close relationship exists between enzyme activity and TG content, there is no direct evidence that the enzyme is interacting with endogenous TG substrate in the myocardium.

Currently, it is thought that LPL is synthesized within the tissue cell and, thereafter, designated for secretion and transport to the capillary beds via the microtubular system (Borensztajn, 1979). This hypothesis suggests that LPL is a highly compartmentalized enzyme. If this is the case, then how does the enzyme gain access to the endogenous TG droplet? Owing to the fact that physical contact must be made between enzyme and substrate before lipolysis can occur, colchicine, which has

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

been shown to block the exit of LPL from the heart cell (Borensztajn *et al.*, 1975), was used as a tool in the present study to demonstrate that lipolysis in heart may be controlled by type L HSL. Another reason for the use of colchicine was that it increased LPL activity without increasing cyclic AMP which, in turn, could possibly activate any one of a number of identified TG lipases in the myocardium (Severson, 1979a).

Methods

Animal care

Male Wistar rats weighing approx. 90g were purchased from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.). The animals were allowed at least 3 weeks to adapt to the living conditions in our animal quarters. Animal room temperature was maintained at $22 \pm 1^\circ\text{C}$ and lighted between 06:00h and 18:00h. Animals were provided free access to a diet of Purina rat chow and water *ad libitum*. Fed animals are defined as rats which had free access to food and water and were killed between 08:00h and 08:30h. Fasted animals are defined as rats that were maintained on their normal diets and then were deprived of food starting at 08:00h. At the time of killing, the rats weighed 200–430g. At that time, the rats were anaesthetized with sodium pentobarbital (5–10mg/100g body wt.) and killed by removing the heart.

Colchicine experiments

At 08:00h on the morning of experimentation, 12 fed-control animals were killed. At the same time, 36 animals received intraperitoneal injections of colchicine (1mg/kg; 5.5mg/ml of saline) and 36 animals were injected with an equivalent volume of saline. Twelve each of colchicine-treated and saline-treated fasted animals were killed at 11:00h, 14:00h and 17:00h.

Triton WR-1339 experiments

At 08:00h on the morning of experimentation, nine fed-control animals were killed. At the same time, nine rats received intravenous injections of 1ml of 0.9% NaCl and nine rats received intravenous injections of 1ml of a 20% (v/v) solution in 0.9% NaCl of Triton WR-1339 (oxyethylated *t*-octylphenol polymethylene polymer; Ruger Chemical Co., Irvington, NJ, U.S.A.). All the Triton-treated and saline-treated fasted animals were killed at 17:00h.

Heart perfusion

Hearts to be analysed for type L HSL activity were isolated and perfused for 2min at a rate of 6ml/min with Krebs–Ringer bicarbonate buffer

(pH 7.4) containing 5 units of heparin/ml to wash LPL and TG from the capillary beds. This procedure was followed by a 2min perfusion using the same buffer without heparin, to wash heparin from the capillary beds. Hearts used for the measurement of total lipase activity were not perfused. The hearts were perfused in a non-recirculatory system as described previously (Oscai, 1979). The perfusion medium was continually gassed with O_2/CO_2 (19:1). The perfusion apparatus was housed in a temperature-regulated acrylic cabinet (Medical Research Apparatus, Clearwater, FL, U.S.A.) in which the temperature was maintained at 37°C . Immediately following perfusion, 100mg of fresh tissue was dissected from the apex of the heart and analysed for lipase activity. The remaining tissue was freeze-clamped with tongs precooled with solid CO_2 /acetone and stored at -80°C until analysis of TG and cyclic AMP.

Chemical assays

Total heart lipase and type L HSL activities were measured by using the LPL assay described by Borensztajn *et al.* (1972). Heart tissue was homogenized in 0.025M- NH_3/HCl buffer (pH 8.1) with a Duall ground-glass tissue grinder (Kontes Glass Co., Evanston, IL, U.S.A.). The tissue concentration of the homogenates was 50mg/ml. Enzyme activity is expressed as units $\pm$ S.E.M.; 1 unit of activity = 1 μmol of non-esterified fatty acid released into the assay medium/h of incubation.

TG was extracted from the heart with ethanol/diethyl ether (3:1, v/v) by the method of Entenman (1957). The concentration of TG was determined as described previously by Fletcher (1968).

Cyclic AMP was determined by the method of Brostrom & Kon (1974) following extraction by the technique described by Weller *et al.* (1972).

Statistical methods

Capillary-bound LPL activity was determined by subtracting type L HSL activity from total heart lipase activity. Results are expressed as means $\pm$ S.E.M. Student's *t* test was used to evaluate the significance of difference between two means. One-way analysis of variance was used to compare the means at the four time points. Significance at $P < 0.05$ warranted the use of the Tukey's *post-hoc* test.

Results

It can be seen in Table 1 that the total amount of LPL activity measured in the hearts of rats deprived of food increased approx. 80% throughout the day. The injection of colchicine did not qualitatively alter this pattern. However, the

colchicine did significantly alter the compartmentalization of the enzyme. While the activity of LPL found in the capillary beds of hearts from saline-injected fasted rats was 86% higher at 17:00h than 08:00h, colchicine treatment prevented this increase. These data suggest that, in colchicine-treated rats, a greater portion of lipase activity is found in the intracellular enzyme fraction (type L HSL) when compared with control.

The results in Fig. 1 indicate that fasting increased type L HSL activity throughout the day. However, at all time points, colchicine significantly potentiated this increase in intracellular lipase activity so that by 17:00h type L HSL activity was 2.3-fold higher than control activity measured at 08:00h.

Table 2 illustrates the effect of fasting + saline and fasting + colchicine injection upon myocardial TG concentration. Throughout the day there was a reduction in heart TG content in the saline-injected fasting rats. Again, colchicine potentiated this response, with TG measured in tissues of colchicine-treated rats being significantly lower at each time point than in the saline-treated group.

Table 1. Effects of fasting and of fasting plus colchicine injection on lipoprotein lipase activity of rat heart
Values are means $\pm$ S.E.M.; $n = 6$ rats for each group.
*Significantly different from total activity measured in 08:00h fed control ($P < 0.001$). †Significantly different from capillary-bound activity measured in 08:00h fed control ($P < 0.01$). ‡Significantly different from capillary-bound activity measured in 17:00h fasted control ($P < 0.01$).

	LPL activity (units/g of tissue) in:	
	08:00h fed	17:00h fasted
Total LPL		
Control	80 $\pm$ 8	140* $\pm$ 9
Colchicine	-	149* $\pm$ 12
Capillary-bound LPL		
Control	28 $\pm$ 6	52† $\pm$ 5
Colchicine	-	23‡ $\pm$ 5

By 17:00h, heart TG content of colchicine-injected rats was only 35% of that measured at 08:00h.

Fig. 2 represents the relationship between cardiac type L HSL activity and TG content. The correlation coefficient of $r = 0.73$ was found to be significant, $P < 0.001$. It is evident from the data given in Table 3 that neither fasting nor fasting + colchicine injection had an effect on the cardiac content of cyclic AMP.

Capillary-bound LPL activity was higher in hearts of control rats compared with that in hearts of colchicine-treated animals measured at 17:00h (Table 1). These higher levels of enzyme activity could have made available to the myocardial cells

Fig. 1. Effect of fasting and fasting + colchicine injection on type L hormone-sensitive lipase activity of rat heart measured throughout the day

Each point represents mean activity $\pm$ S.E.M. All activities are significantly greater than the activity at 08:00h ($P < 0.01$). Activities measured in colchicine-injected rat hearts are significantly ($P < 0.01$) greater than those of saline-injected rats at 11:00h, 14:00h and 17:00h. —, Colchicine-injected; ---, saline-injected.

Table 2. Effects of fasting and of fasting plus colchicine injection on endogenous triacylglycerol content in rat heart
Values are means $\pm$ S.E.M.; $n = 6$ rats for each group. *Significantly different from TG content measured in 08:00h fed control ($P < 0.001$). †Significantly different from TG content in fasted control rats measured at the same time ($P < 0.001$).

	TG content (μ mol/g of tissue) in:			
	08:00h fed	11:00h fasted	14:00h fasted	17:00h fasted
Control	1.73 $\pm$ 0.02	1.57* $\pm$ 0.02	1.51* $\pm$ 0.03	1.35* $\pm$ 0.01
Colchicine	-	1.22*† $\pm$ 0.01	0.98*† $\pm$ 0.02	0.58*† $\pm$ 0.02

Fig. 2. Relationship between type L hormone-sensitive lipase activity and triacylglycerol content in hearts of fasted and fasted + colchicine-treated rats

The line corresponds to $y = -0.001x + 2.06$
($r = -0.73$, $P < 0.001$).

more non-esterified fatty acids for endogenous TG synthesis to account for the higher TG values found in hearts of control rats (versus colchicine-treated animals, Table 2). In an attempt to rule out this possibility, an experiment was carried out in which rats were injected intravenously with Triton WR-1339 at 08:00h while control animals were injected with an equivalent volume of saline. Triton WR-1339 is a non-ionic detergent which has been shown to inhibit selectively that LPL which is directly involved in the hydrolysis of circulating TG *in vivo* (Borensztajn *et al.*, 1976). As expected, at 17:00h the capillary-bound LPL was decreased from 45 ± 7 units/g of tissue to 11 ± 2 units/g of tissue. Under these conditions, endogenous TG content of the myocardium was increased in the Triton-treated rats from $1.46 \pm 0.06 \mu\text{mol/g}$ of tissue to $2.05 \pm 0.03 \mu\text{mol/g}$ of tissue. These results suggest that the decrease in cardiac TG content seen in the colchicine-treated rats in Table 2 is not due to the lower capillary-bound LPL value in colchicine-treated rats (23 ± 5 units/g) compared with that in control rats (52 ± 5 units/g) measured at 17:00h. At a time when cardiac TG content was increased in the Triton-treated rats, intracellular LPL was reduced

from 87 ± 5 units/g of tissue to 60 ± 4 units/g of tissue. These results are consistent with our working hypothesis that type L HSL may play a role in the regulation of myocardial lipolysis.

Discussion

A number of lipases have been implicated in the hydrolysis of endogenous TG in heart. Bjorntorp & Furman (1962) reported that cardiac muscle contained two lipases, one with characteristics of LPL and the other with a neutral pH optimum. Further, the neutral lipase, but not the alkaline lipase, was found to be resistant to NaCl and protamine. In other early studies, high activity of a cardiac TG lipase has been noted in the microsomal fraction (George & Iype, 1963; Biale *et al.*, 1968; Shimoda, 1968; Hamid *et al.*, 1973). Mersel *et al.* (1979) reported TG lipase activities with two optimum pH values (pH 7.4 and 8.5) for heart. Schousboe *et al.* (1973) reported that 50% of TG lipase activity from heart was in the soluble fraction and the other half evenly distributed between the microsomal and mitochondrial fractions. LPL was present in all fractions as judged by the effects of serum, NaCl, and heparin. Glaviano *et al.* (1975) found that noradrenaline infused in open-chest dogs activated a myocardial TG lipase. Jesmok *et al.* (1976) reported the existence of a TG lipase from 100000g supernatant of ventricular extracts treated with Lubrol-PX. Wang *et al.* (1977) identified a cardiac TG lipase of lysosomal origin in an assay system buffered at pH 8.0. Rosen *et al.* (1981) identified an acid and neutral TG lipase in rat heart. The pH-dependency of the lipases revealed maximum activity at pH 4.5 and between 7.0 and 7.5. Hulsmann *et al.* (1981) also provided evidence for acid (pH 5) and neutral (pH 7.0) lipases hypothesized to be involved in endogenous lipolysis in heart. Murthy *et al.* (1983) reported the existence of an ATP-sensitive TG lipase measured in 100000g pellet from rat heart. Severson (1979b) measured LPL activity in the 100000g supernatant fraction from rat heart homogenates as judged by a serum requirement, alkaline pH, and inhibition by NaCl. In addition

Table 3. Effects of fasting and of fasting plus colchicine injection on cyclic AMP concentration in rat heart
Values are means $\pm$ S.E.M.; $n = 6$ rats for each group.

	Cyclic AMP content (nmol/g of tissue)				
	Perfused				Non-perfused
	08:00h fed	11:00h fasted	14:00h fasted	17:00h fasted	17:00h fasted
Control	0.227 ± 0.011	0.226 ± 0.072	0.248 ± 0.071	0.256 ± 0.022	0.252 ± 0.024
Colchicine	—	0.195 ± 0.045	0.216 ± 0.027	0.254 ± 0.020	0.261 ± 0.022

to LPL, he identified TG lipase activity with both an acid (pH 4.5–5) and neutral (pH 7.5) pH optimum. The acid TG lipase was localized to particulate subcellular fractions, whereas neutral lipase activity was present in both soluble and particulate fractions. Preincubation of the rat heart preparations with cyclic AMP and protein kinase resulted in no consistent effect on lipase activity determined at either pH 5 or 7.5 (Severson, 1979b). Collectively, the identified cardiac TG lipases have been classified according to their cellular location and/or pH optimum. The Achilles' heel of the above work has been an inability to demonstrate the activation of a TG lipase with cyclic AMP or protein kinase in the heart (Lech *et al.* 1977). This is an important consideration, because it has been long recognized that cyclic AMP may mediate catecholamine- and glucagon-stimulated lipolysis in heart (Gartner & Vahouny, 1972; Crass, 1973; Lech *et al.*, 1977).

Colchicine, which is known to interfere with the integrity of the microtubular system (Borisy & Taylor, 1967), has no effect on LPL activity *per se* (Borensztajn *et al.*, 1975). However, this drug has been shown to block the intracellular transport of various hormones, glycoproteins, and lipoproteins (Lacy *et al.*, 1968; Rossignol *et al.*, 1972; Pelletier & Bornstein, 1972; Williams & Wolff, 1972; Marchand *et al.*, 1973; Stein & Stein, 1973; Stein *et al.*, 1974; Douglas & Sorimachi, 1972), as well as LPL (Borensztajn *et al.*, 1975; Chajek *et al.*, 1975).

LPL activity in heart muscle is low in the morning after overnight feeding but increases throughout the day in rats that have been deprived of food starting at approx. 08:00 h (Borensztajn *et al.*, 1975). In the present study, intracellular LPL activity increased in hearts of fasting rats. This increase in activity was potentiated in colchicine-treated animals as a result of blocking enzyme export from the cell. Since endogenous TG stores were decreased at the same time that intracellular LPL activity was increased, these results provide indirect evidence that type L HSL is 'seeing the endogenous TG droplet'. Adding support to this interpretation is the observation that, at a time when TG hydrolysis was occurring, cyclic AMP levels remained unchanged, ruling out the possibility that cyclic AMP might be activating one of the above mentioned TG lipases. In earlier work (Oscai, 1979; Palmer *et al.*, 1981a; Palmer & Kane, 1983a,b), enzyme activity was increased with agents that produced increases in the cellular concentration of cyclic AMP. Using that method of enzyme activation, a high negative correlation ($r > -0.90$) exists between type L HSL activity and endogenous TG concentration. In the present study, we have employed two techniques that elevate type L HSL activity without modifying the

intracellular cyclic AMP content. It has been reported that there is no diurnal rhythm in the heart cyclic AMP concentration of fed rats (Palmer *et al.*, 1981b). Table 2 indicates that fasting or fasting+colchicine treatment did not affect myocardial cyclic AMP concentration. Moreover, fasting and fasting+colchicine treatment increased type L HSL activity independent of other tissue lipases. This protocol produced a significant negative relationship between lipase activity and TG content ($r = -0.73$). These data are consistent with the hypothesis that type L HSL is playing a significant role in cardiac lipolysis.

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References

- Biale, Y., Gorin, E. & Shafir, E. (1968) *Biochim. Biophys. Acta* **152**, 28–39
- Bjorntorp, P. & Furman, R. H. (1962) *Am. J. Physiol.* **203**, 323–326
- Borensztajn, J. (1979) in *The Biochemistry of Atherosclerosis* (Scanu, A. M., Wissler, R. W. & Getz, G. S., eds.), pp. 231–245, Marcel Dekker, New York
- Borensztajn, J., Samols, D. R. & Rubenstein, A. H. (1972) *Am. J. Physiol.* **223**, 1271–1275
- Borensztajn, J., Rone, M. S. & Sandros, T. (1975) *Biochim. Biophys. Acta* **398**, 394–400
- Borensztajn, J., Rone, M. S. & Kotlar, T. J. (1976) *Biochem. J.* **156**, 539–543
- Borisy, G. G. & Taylor, E. W. (1967) *J. Cell Biol.* **34**, 525–533
- Brostrom, C. O. & Kon, C. (1974) *Anal. Biochem.* **58**, 459–468
- Chajek, T., Stein, O. & Stein, Y. (1975) *Biochim. Biophys. Acta* **380**, 127–131
- Crass, M. F. (1973) *Recent Adv. Stud. Card. Struct. Metab.* **3**, 275–289
- Douglas, W. W. & Sorimachi, M. (1972) *Br. J. Pharmacol.* **45**, 129–132
- Entenman, C. (1957) *Methods Enzymol.* **3**, 301–302
- Fletcher, M. J. (1968) *Clin. Chim. Acta.* **22**, 393–397
- Gartner, S. L. & Vahouny, G. V. (1972) *Am. J. Physiol.* **222**, 1121–1124
- George, J. C. & Iype, P. T. (1963) *Am. J. Physiol.* **204**, 165–167
- Glaviano, V. V., Goldberg, J. & Pindok, M. T. (1975) *Am. J. Physiol.* **228**, 1678–1684
- Hamid, M. A., Lech, J. J., Barboriak, J. J. & Calvert, D. N. (1973) *Biochem. Pharmacol.* **22**, 2165–2170
- Hulsmann, W. C., Stam, H. & Breeman, W. A. P. (1981) *Biochem. Biophys. Res. Commun.* **102**, 440–448
- Jesmok, G. J., Mogelnicki, S. R., Lech, J. J. & Calvert, D. N. (1976) *J. Mol. Cell. Cardiol.* **8**, 283–298

- Lacy, P. E., Howell, S. L., Young, D. A. & Fink, C. J. (1968) *Nature (London)* **219**, 1177-1179
- Lech, J. J., Jesmok, G. J. & Calvert, D. N. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 2000-2008
- Marchand, Y. L., Singh, A., Assimacopoulos-Jeannet, F., Orci, L., Rouiller, C. & Jeanrenaud, B. (1973) *J. Biol. Chem.* **248**, 6862-6870
- Mersel, M., Heller, M. & Pinson, A. (1979) *Biochim. Biophys. Acta* **572**, 218-224
- Miller, W. C. & Oscai, L. B. (1984) *Am. J. Physiol.*, in the press
- Murthy, V. K., Stiles, M. R. & Shipp, J. C. (1983) *Lipids* **18**, 408-411
- Oscai, L. B. (1979) *Biochem. Biophys. Res. Commun.* **91**, 227-232
- Oscai, L. B., Caruso, R. A. & Wergeles, A. C. (1982) *J. Appl. Physiol.: Respir. Environ. Exercise Physiol.* **52**, 1059-1063
- Palmer, W. K. & Kane, T. A. (1983a) *Biochem. J.* **212**, 379-383
- Palmer, W. K. & Kane, T. A. (1983b) *Biochem. J.* **216**, 241-243
- Palmer, W. K., Caruso, R. A. & Oscai, L. B. (1981a) *Biochem. J.* **198**, 159-166
- Palmer, W. K., Studney, T. A. & Doukas, S. (1981b) *Biochim. Biophys. Acta* **672**, 114-122
- Pelletier, G. & Bornstein, M. B. (1972) *Exp. Cell. Res.* **70**, 221-223
- Robinson, D. S. (1970) *Compr. Biochem.* **18**, 51-116
- Rosen, P., Budde, T. & Reinauer, H. (1981) *J. Mol. Cell. Cardiol.* **13**, 539-550
- Rosignol, B., Herman, G. & Keryer, G. (1972) *FEBS Lett.* **21**, 189-194
- Schousboe, I., Bartels, P. D. & Jensen, P. K. (1973) *FEBS Lett.* **35**, 279-283
- Severson, D. L. (1979a) *Can. J. Physiol. Pharmacol.* **57**, 923-937
- Severson, D. L. (1979b) *J. Mol. Cell. Cardiol.* **11**, 569-583
- Shimoda, M. (1968) *Jpn. Circ. J.* **32**, 813-817
- Stein, O. & Stein, Y. (1973) *Biochim. Biophys. Acta* **306**, 142-147
- Stein, O., Sanger, L. & Stein, Y. (1974) *J. Cell Biol.* **62**, 90-103
- Wang, T.-W., Menahan, L. A. & Lech, J. J. (1977) *J. Mol. Cell. Cardiol.* **9**, 25-38
- Weller, M., Rodnight, R. & Carrera, D. (1972) *Biochem. J.* **129**, 113-121
- Williams, J. A. & Wolff, J. (1972) *J. Cell Biol.* **54**, 157-165