Hormonal activation of type-L hormone-sensitive lipase measured in defatted heart powders

Warren K. PALMER and Therese A. KANE

Department of Physical Education, University of Illinois at Chicago, Box 4348, Chicago, IL 60680, U.S.A.

(Received 25 November 1982/Accepted 10 February 1983)

Adrenaline, 3-isobutyl-1-methylxanthine (MIX) and dibutyryl cyclic AMP (Bt₂ cyclic AMP) stimulated type-L hormone-sensitive lipase (HSL) activity when measurements were made on defatted rat heart powders. These lipolytic agents stimulated the activity of this enzyme in a time- and dose-dependent manner. This activation was reversible, because removal of adrenaline from the perfusate was accompanied by the return of type-L HSL activity to control levels. We have reported [Palmer, Caruso & Oscai (1981) *Biochem. J.* **198**, 159–166] that perfusion with low levels of adrenaline, MIX or Bt₂ cyclic AMP reduced type-L HSL activity below control levels when measurements were made in aqueous homogenates. However, in the present study, when activities were measured in acetone/diethyl ether heart powders, all concentrations of these agents studied stimulated enzyme activity, and at no concentration was there enzyme inhibition. These data suggest that acetone/diethyl ether treatment may remove a factor that plays a role in type-L HSL regulation. Type-L HSL activity measured in acetone/diethyl ether powders of control and stimulated rat heart exhibited properties that include alkaline pH optimum, serum requirement, activation by heparin and inhibition by high salt and protamine sulphate. These characteristics, in addition to the stability of the enzyme to treatment with organic solvents, fulfil the requirements for the type-L HSL classification.

Hormonal stimulation of cardiac lipolysis is well documented (Gartner & Vahouny, 1972; Crass *et al.*, 1975). Recently, we showed that the heparin non-releasable fraction, hypothesized to be the intracellular fraction (Borensztajn *et al.*, 1975), of lipoprotein lipase was activated and inhibited by cyclic AMP (Palmer *et al.*, 1981). High concentrations of agent stimulated enzyme activity, whereas low concentrations of nucleotide inhibited lipase activity. The activity of this enzyme related closely to both the level of intracellular triacylglycerol, as well as the concentration of non-esterified fatty acid in the tissue. The activity, measured in crude aqueous heart homogenate, was sensitive to heparin, protamine sulphate and NaCl. Control and stimulated lipase activities had a serum requirement and a pH optimum of 8.1. Since there are no plasma lipoproteins in heart parenchymal cells, and because hormone sensitivity has been demonstrated, this lipase has been termed type-L (having characteristics of the lipoprotein lipase) HSL (Palmer, 1983).

Abbreviations used: HSL, hormone-sensitive lipase; MIX, 3-isobutyl-1-methylxanthine; Bt₂ cyclic AMP, N⁶O²-dibutyryl cyclic AMP.

Lipoprotein lipase (LPL) is stable to treatment with acetone and diethyl ether (Robinson, 1970). In fact, investigators (Salaman & Robinson, 1966; Borensztajn *et al.*, 1972; Huttunen *et al.*, 1972) have taken advantage of this property by using it to characterize LPL and separate this enzyme from other organic-solvent-labile lipases. Since previous measurements on activated type-L HSL have been performed on aqueous homogenates (Oscai, 1979; Palmer *et al.*, 1981), it was the primary purpose of the present experiments to determine if stimulation of type-L HSL with lipolytic agents could be detected when perfused hearts were defatted by acetone/diethyl ether treatment. The results obtained remain consistent with the 'L' classification of this HSL. An additional objective of the present work was to determine if the hormonal modification of enzyme activity was reversible when the agent was removed from the perfusate.

Experimental

Animal care

Male Wistar rats weighing approx. 90g were purchased from Charles River (Wilmington, MA,

U.S.A.). Animals were provided access to a diet of Purina rat chow and water *ad libitum*. The animals were allowed at least 3 weeks to adapt to the living conditions in our animal quarters. Animal room temperatures were maintained at $22 \pm 1^\circ\text{C}$ and lighted between 07:00 and 19:00h. To control for the circadian rhythms in cardiac triacylglycerol lipase activity, all experiments were performed between 08:00h and 10:00h (Kotlar & Borensztajn, 1977). At the time of killing, the rats weighed approx. 230g. After overnight starvation, the rats were anaesthetized with sodium pentobarbital (60mg/kg body wt.) and killed by removing the heart.

Heart perfusion and tissue preparation

Hearts were isolated and perfused for 4 min at a rate of 6 ml/min with Krebs–Ringer bicarbonate (KRB) buffer (pH 7.4) containing 5% (v/v) calf serum (Gibco, New York, NY, U.S.A.) and 5 units of heparin/ml, to remove lipoprotein lipase from the capillary beds. At the end of the 4 min perfusion, the perfusates were free of lipoprotein lipase. The hearts were then perfused with KRB buffer containing either adrenaline, MIX (Aldrich Chemical Co., Milwaukee, WI, U.S.A.) or Bt_2 cyclic AMP (Sigma Chemical Co., St. Louis, MO, U.S.A.). Hearts were then perfused for 2 min with the original buffer (minus heparin) to wash the stimulating agent from the capillary beds. Control hearts were perfused in a similar fashion without the use of stimulating agent.

At the end of the perfusion period, hearts were weighed, minced and homogenized in 20 vol. of cold acetone by using a Polytron homogenizer. The homogenate was filtered through Whatman filter paper (no. 1) and subsequently washed with 150 ml of cold acetone, 250 ml of room-temperature acetone and 250 ml of room-temperature diethyl ether. Filter papers containing the heart powders were then freeze-dried for 30 min.

Lipase assay

Enzyme activity was measured by using the classical lipoprotein lipase assay described by Borensztajn *et al.* (1972). Defatted heart powders were homogenized (10 mg/ml) in 25 mM- NH_3/HCl buffer (pH 8.1) with a Duall ground-glass tissue grinder (Kontes Glass Co., Evanston, IL, U.S.A.). Type-L HSL activities are expressed as units $\pm$ S.E.M.; 1 unit of activity released $1 \mu\text{mol}$ of non-esterified fatty acid into the assay medium/h of incubation. The assays were linear with time and protein content regardless of tissue treatment.

Results

The lipolytic agents adrenaline, MIX and Bt_2 cyclic AMP stimulated type-L HSL activity even

when measurements were made on defatted rat heart powders (Figs. 1 and 2; Table 1). Fig. 1 shows that adrenaline stimulated type-L HSL activity of perfused hearts when hearts were assayed after treatment with organic solvents. The activation is time-dependent, with maximal stimulation being attained at 15 min of perfusion when $0.005 \mu\text{M}$ -adrenaline was utilized. When higher concentrations of adrenaline were used, maximal activity was attained more rapidly than shown in Fig. 1. For example, $1.0 \mu\text{M}$ -adrenaline fully stimulated type-L HSL activity with only 3 min of perfusion. When less than $0.005 \mu\text{M}$ -adrenaline was contained in the perfusate, type-L HSL activity reached a plateau at a lower level than that seen in Fig. 1 (approx. 450 units/g of powder).

When adrenaline was removed from the flow-through perfusion system, at 15 min, the activity returned to control levels by 45 min of perfusion. Restimulation of the perfused heart with adrenaline again stimulated type-L HSL to the same activity that was attained when the heart was perfused continuously for 50 min with adrenaline.

When hearts were perfused with various amounts of the cyclic AMP phosphodiesterase inhibitor, MIX, type-L HSL activity, measured in defatted heart powders, was stimulated in a dose-dependent manner until a perfusate concentration of $230 \mu\text{M}$ was reached (Table 1). Increasing the amount of MIX did not increase enzyme activity to any greater extent. Fig. 2 shows that when $230 \mu\text{M}$ -xanthine was

Fig. 1. Type-L HSL activity measured in acetone/diethyl ether preparations of perfused rat heart

The hatched area denotes perfusion time with $0.005 \mu\text{M}$ -adrenaline. ●, Mean ($\pm$ S.E.M.) type-L HSL activity in stimulated hearts; ▲, activities of hearts perfused without adrenaline. The numbers of hearts analysed at each time point are given in parentheses. ○, Mean activity measured in powders of two hearts perfused with adrenaline continuously for 50 min.

Table 1. Type-L HSL activity measured in defatted powders of hearts perfused for 15 min with various amounts of MIX

All values are means $\pm$ S.E.M. Numbers of hearts per concentration are given in parentheses.

Dose (μ M)	Type-L HSL activity (units/g of powder)
None	280 $\pm$ 7 (20)
11	360 $\pm$ 9 (3)
23	406 $\pm$ 13 (4)
45	416 $\pm$ 20 (6)
230	465 $\pm$ 31 (5)
450	440 $\pm$ 34 (3)
3150	402 $\pm$ 12 (7)

Fig. 2. Type-L HSL activity is measured in acetone/diethyl ether preparations of rat heart perfused for various lengths of time with either 230 μ M-MIX (●) or 10 μ M-Bt₂ cyclic AMP (○)

▲, Indicates activities of hearts perfused without stimulating agent. The numbers of hearts analysed at each time point are given in parentheses.

used, the time-course of type-L HSL activation was similar to that for the adrenaline concentration shown in Fig. 1. By 15 min, the enzyme was fully activated, with no further increase occurring for up to 30 min of perfusion with the agent.

When submaximal amounts of adrenaline and MIX were combined in the perfusion medium, an additive stimulation of type-L HSL took place. Three experiments of this type were performed. Perfusion for 15 min with 0.0025 μ M-adrenaline stimulated type-L HSL activity from the control level of 278 $\pm$ 21 units/g of powder to 357 $\pm$ 12 units/mg of powder (mean $\pm$ S.E.M.). Perfusion with 11 μ M-MIX increased enzyme activity to 353 $\pm$ 19 units/g of powder. Combining 0.0025 μ M-adrenaline with a 11 μ M concentration of MIX for 15 min

of perfusion stimulated type-L HSL activity to 417 $\pm$ 11 units/g of powder.

Perfusion of the hearts with Bt₂ cyclic AMP stimulated type-L HSL activity in a dose-dependent manner. The data in Fig. 2 show the time-course of enzyme activation when perfusing with 10 μ M-Bt₂ cyclic AMP. Enzyme activation was more rapid than that seen for adrenaline (Fig. 1) or MIX (Fig. 2).

When the type-L HSL was measured in whole homogenates, lipolytic agents had a biphasic effect on enzyme activity (Palmer *et al.*, 1981). In Table 2, it is evident that a different response is seen when assays are performed on acetone/diethyl ether powders than when analyses are made on aqueous homogenates. Table 2 has been constructed from data published previously (Palmer *et al.*, 1981) and analysis of acetone/diethyl ether preparations of rat heart. Perfusion with low levels of lipolytic agent significantly decreased type-L HSL activity when measurements were made on aqueous homogenates. However, when hearts were perfused with the same concentrations of adrenaline, MIX or Bt₂ cyclic AMP, and measurements made on acetone/diethyl ether powders, enzyme activity was stimulated. In fact, at no time have we experienced an inhibitory influence by lipolytic agent on type-L HSL activity when measurements were made on defatted heart powders.

Table 2. The effect of adrenaline, MIX and Bt₂ cyclic AMP perfusion on the activity of type-L HSL measured in aqueous homogenates or defatted powders of rat heart

All values are means $\pm$ S.E.M. Numbers of hearts per group are given in parentheses. The data obtained for aqueous homogenates came from the work published in Palmer *et al.* (1981). Although the perfusion conditions differed slightly in that work (30 min rather than 15 min), the activities measured for acetone/diethyl ether preparation of heart were the same at 15 and 30 min (see Fig. 2).

Agent	Type-L HSL activity	
	Whole homogenate (units/g wet wt.)	Defatted powder (units/g of powder)
None	73 $\pm$ 0.2 (8)	280 $\pm$ 7 (20)
Adrenaline (0.005 μ M)	22 $\pm$ 0.2* (4)	438 $\pm$ 14† (7)
MIX (23 μ M)	11 $\pm$ 0.5* (5)	406 $\pm$ 13† (7)
Bt ₂ cyclic AMP (5 μ M)	22 $\pm$ 1.0* (8)	453 $\pm$ 32† (3)

* Significantly less than control aqueous homogenate activity ($P < 0.001$).

† Significantly greater than control defatted powder activity ($P < 0.001$).

To determine if the control and stimulated type-L HSL activity exhibited properties similar to that seen in aqueous heart homogenate, we characterized the control and stimulated type-L HSL activity measured in defatted heart powders. The data in Table 3 show the effect of inclusion of 1 M-NaCl or 1 mg of protamine sulphate/ml in the assay. In addition, the dependence of enzyme activity on serum was determined. Removal of serum from the assay was associated with greater than 90% decrease in enzyme activity regardless of whether the hearts were stimulated or non-stimulated. Inclusion of 1 M-NaCl in the assay showed a similar inhibitory effect. Protamine sulphate addition to the assay reduced enzyme activity found in acetone/diethyl ether heart powders of control and stimulated hearts

Table 3. *Effects of serum, NaCl and protamine sulphate on type-L HSL activity in defatted powders of hearts perfused with adrenaline, MIX or Bt₂ cyclic AMP*

Additions ...	Type-L HSL activity (units/g of powder)			
	Normal assay	-Serum	NaCl (1 M)	Protamine sulphate (1 mg/ml)
Control	295	25	16	56
Adrenaline (0.005 μ M)	444	15	9	44
MIX (230 μ M)	436	16	11	45
Bt ₂ cyclic AMP (10 μ M)	452	23	16	30

to approximately the same level of activity (approx. 45 units/g of powder).

It can be seen in Table 4 that only 53% of the control activity could be measured when no heparin was present in the assay. When hearts were stimulated with adrenaline, MIX or Bt₂ cyclic AMP, removal of heparin from the assay decreased activity 41%, 57% and 54% respectively. Inclusion of a 4-fold increase in heparin in the assay was associated with approximately a 20% reduction in type-L HSL activity in control samples and less than a 10% reduction in activity in the stimulated rat heart powders. Maximal type-L HSL activity was measured when 1 unit of heparin/ml was in the assay.

It is evident from the data in Table 5 that enzymic activity could be measured in a wide pH range. However, maximal activity was measured at a pH optimum of 8.1 in the acetone/diethyl ether powders of control and stimulated hearts. At a pH of 7.4 adrenaline only increased enzymic activity 18% above control values. When measurements were made at pH 8.1, adrenaline increased type-L HSL activity 40%.

Discussion

Recently, evidence has been provided suggesting that the intracellular fraction of lipoprotein lipase plays a role in the hydrolysis of endogenous triacylglycerols in the rat heart. Borensztajn *et al.* (1973) reported that injection of glucagon into the rat increased lipoprotein lipase activity in whole

Table 4. *The effect of heparin on the type-L HSL activity in defatted powders of hearts perfused with adrenaline, MIX or Bt₂ cyclic AMP*

Heparin concentration (units/ml) ...	Type-L HSL activity (units/g of powder)					
	0	0.5	1*	2	3	4
Control	159	286	297	248	244	238
Adrenaline (0.005 μ M)	250	404	422	412	405	396
MIX (230 μ M)	191	379	444	415	410	408
Bt ₂ cyclic AMP (10 μ M)	213	405	461	452	437	418

* Concentration of heparin in assay described in the Experimental section.

Table 5. *The effect of pH on type-L HSL activity in defatted powders of hearts perfused with adrenaline, MIX or Bt₂ cyclic AMP*

pH ...	Type-L HSL activity (units/g of powder)					
	7.4	7.8	8.1*	8.3	8.5	9.0
Control	218	239	292	263	252	233
Adrenaline (0.005 μ M)	258	321	407	374	334	301
MIX (230 μ M)	280	230	430	400	385	268
Bt ₂ cyclic AMP (10 μ M)	259	323	440	411	380	368

* pH of assay described in the Experimental section.

heart homogenates. Oscai (1979) showed that there was increased activity in the heparin-non-releasable fraction of aqueous heart homogenates. The elevated enzyme activity was accompanied by a reduction in cardiac triacylglycerol concentration. Subsequently, Palmer *et al.* (1981) found that agents that elevate [cyclic AMP] have a biphasic effect on lipoprotein lipase activity of heparin-perfused hearts. Moreover, enzyme activity in aqueous homogenates correlated well with myocardial triacylglycerol and non-esterified fatty acid content. The activated enzyme had properties of lipoprotein lipase. Therefore, since it was possible to demonstrate hormone sensitivity and because plasma lipoproteins do not exist in heart parenchymal cells, this intracellular lipase has been termed type-L HSL.

In the present study, it was possible to demonstrate hormone sensitivity of type-L HSL. However, the biphasic effect of lipolytic agents disappears when hearts are delipidated with organic solvents. Removal of the inhibitory effect of low doses of lipolytic agent by treatment with acetone/diethyl ether may suggest that a type-L HSL inhibitor is being removed that may play a role in the regulation of this enzyme.

Sutherland has proposed a list of criteria for determining when a hormone-induced event is mediated by cyclic AMP (Robison *et al.*, 1971). In the present study, stimulation of type-L HSL activity by adrenaline, a hormone shown to increase cardiac cyclic AMP levels (Robison *et al.*, 1968), and by perfusion with Bt₂ cyclic AMP fulfil two of Sutherland's criteria. When type-L HSL activity was measured in acetone/diethyl ether powders of hearts perfused with submaximal amounts of adrenaline and MIX, these two agents yielded higher activity than could be elicited by either agent alone. The synergistic potentiation of the hormone's effect by administration of the cyclic AMP phosphodiesterase inhibitor suggests that the activation of type-L HSL is mediated directly or indirectly by cyclic AMP.

In 1955, Korn (1955a) identified specific characteristics of an acetone-stable lipase in the heparin non-perfused rat heart. These characteristics are now used to identify lipoprotein lipase. These properties included enzyme sensitivity to heparin, high salt and protamine. Korn & Quigley (1957) determined that the pH optimum for the enzyme activity was 8.1. Serum (Korn, 1955b), and particularly serum apolipoprotein C-II (Lukens & Borensztajn, 1978), has been identified as a potent stimulator of lipoprotein lipase. We have used these properties to characterize a hormone-stimulated lipase identified in heparin-perfused (intracellular fraction) rat heart. Activity measured in acetone/diethyl ether powders from control hearts and hearts stimulated with the lipolytic agents adrenaline,

MIX and Bt₂ cyclic AMP exhibited the common properties, including an alkaline pH optimum, serum requirement, activation by heparin and inhibition by high salt and protamine sulphate. Since hormone stimulation of lipase activity could be identified in acetone/diethyl ether powders of rat heart, and because the elevated activity had the characteristics described, the findings reported in the present paper are consistent with the type-L designation of this HSL.

Although the concept has developed that lipoprotein lipase functions exclusively as a clearing factor responsible for the hydrolysis of triacylglycerol in the circulation (Robinson, 1970; Borensztajn, 1975), it is becoming apparent that the intracellular fraction of lipoprotein lipase (type-L HSL) functions as a regulator of triacylglycerol concentration in the rat myocardium. The action of lipolytic agents on this enzyme seems to be mediated directly or indirectly through cyclic AMP.

We thank Mrs. Mary Ann Fritsch for manuscript preparation. This work was supported by grants from the Chicago Heart Association and from the National Institutes of Health HL 26435.

References

- Borensztajn, J. (1975) 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., Keig, P. & Rubenstein, A. H. (1973) *Biochem. Biophys. Res. Commun.* **53**, 603–608
- Borensztajn, J., Rone, M. S. & Sandros, T. (1975) *Biochim. Biophys. Acta* **398**, 394–400
- Crass, M. F., Shipp, J. C. & Pieper, G. M. (1975) *Am. J. Physiol.* **228**, 618–627
- Gartner, S. L. & Vahouny, G. V. (1972) *Am. J. Physiol.* **222**, 1121–1124
- Huttunen, J. K., Ellingboe, J., Pittman, R. C. & Steinberg, D. (1972) *Biochim. Biophys. Acta* **218**, 333–346
- Korn, E. D. (1955a) *J. Biol. Chem.* **215**, 1–4
- Korn, E. D. (1955b) *J. Biol. Chem.* **215**, 15–27
- Korn, E. D. & Quigley, T. W., Jr. (1957) *J. Biol. Chem.* **226**, 833–839
- Kotlar, T. J. & Borensztajn, J. (1977) *Am. J. Physiol.* **233**, E316–E319
- Lukens, T. W. & Borensztajn, J. (1978) *Biochem. J.* **175**, 1143–1146
- Oscai, L. B. (1979) *Biochem. Biophys. Res. Commun.* **91**, 227–232
- Palmer, W. K. (1983) *Med. Sci. Sports Exercise* in the press
- Palmer, W. K., Caruso, R. A. & Oscai, L. B. (1981) *Biochem. J.* **198**, 159–166
- Robinson, D. S. (1970) in *Comprehensive Biochemistry* (Florkin, M. & Stotz, E. H., eds.), pp. 51–116, Elsevier Publishing Co., New York
- Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1968) *Annu. Rev. Biochem.* **37**, 149–174
- Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1971) *Cyclic AMP*, p. 63, Academic Press, New York
- Salaman, M. R. & Robinson, D. S. (1966) *Biochem. J.* **99**, 640–647