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ACID AND NEUTRAL TRIACYLGLYCEROL ESTER HYDROLASES IN RAT HEART

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SUMMARY

Subcellular fractions from rat heart homogenates can be characterized as having acid and neutral triacylglycerol ester hydrolase (lipase) activities in addition to lipoprotein lipase activity. Perfusion of rat hearts with heparin resulted in a decrease in lipoprotein lipase activity but no change in neutral triacylglycerol ester hydrolase activity, indicating that these two lipase activities probably represent separate and distinct enzymes. A comparison of the subcellular distribution of the acid triacylglycerol ester hydrolase with a lysosomal marker enzyme (N-acetylglucosaminidase) suggests that the acid lipase originated in cardiac lysosomes.

INTRODUCTION

Free fatty acids are the preferred oxidative substrate for the myocardium. These free fatty acids can be present in the circulation as a consequence of lipolysis at adipose tissue (1), or be derived from the hydrolysis of lipoprotein-bound triacylglycerols by lipoprotein lipase situated at the capillary endothelium of the heart (2-4). In addition, the hydrolysis of endogenous triacylglycerols by a lipase present within the myocardial cell can also support the energy requirements of the heart under certain circumstances (1).

Previous studies on the properties of cardiac lipases have identified lipoprotein lipase activity plus various "tissue lipase(s)" which have usually been characterized as having a neutral or alkaline pH optimum (5-9). In contrast, Hülsmann and Stam have concluded from heparin-perfusion experiments that rat hearts contain only an acid triacylglycerol lipase in addition to lipoprotein lipase (10). A recent study from this laboratory has identified lipoprotein lipase and both acid (pH optimum of 5) and neutral (pH optimum of 7.5) triacylglycerol ester hydrolase (lipase) activities in subcellular fractions from rat heart homogenates (11). The present investigations were undertaken to further distinguish and characterize these cardiac lipase activities.

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METHODS

Rat hearts were homogenized in 10 vol. of Buffer A (0.25 M sucrose, 10 mM HEPES, 1 MM EDTA, pH 7.5) for 30 sec. with a Polytron tissue homogenizer. The resulting homogenate was centrifuged for 20 min. at 400 x g to remove cellular debris, and the supernatant was carefully removed. The pellet was resuspended in Buffer A and re-centrifuged at 400 x g; the supernatants from the two low-speed spins were then combined. In some experiments, subcellular fractions were obtained by differential centrifugations at 5,000 x g, 17,000 x g and 100,000 x g.

For the perfusion experiments, rat hearts were perfused retrograde with Krebs-Henseleit bicarbonate buffer containing 5.5 mM D-glucose as described previously (12), prior to the homogenization and preparation of subcellular fractions.

Lipoprotein lipase activity was determined with an ethanolic triolein substrate preparation as described by Severson (11). Acid triacylglycerol ester hydrolase activity (measured at pH 5 with an acetate buffer) and neutral triacylglycerol ester hydrolase activity (measured at pH 7.5 with either phosphate or PIPES buffers) was determined in assays with either an ethanolic triolein (11) or glycerol-dispersed triolein (13) substrate preparation. A unit of hydrolase (lipase) activity was arbitrarily defined as that amount of enzyme which catalyzed the formation of 1 nmol of free fatty acid (oleate) in 1 h at 30°C. N-acetylglucosaminidase activity was assayed at pH 4 according to the procedure of Peters *et al* (14).

RESULTS

Lipoprotein lipase can be characterized by specific *in vitro* criteria such as serum activation and an alkaline pH optimum (1-4,11). Previously, a neutral triacylglycerol ester hydrolase activity in rat heart was demonstrated under experimental conditions in which the contribution of lipoprotein lipase to the hydrolase assay was minimal. Since perfusion of rat hearts with heparin will remove a portion of the total lipoprotein lipase (15), the effect of heparin perfusion on lipoprotein lipase and neutral triacylglycerol ester hydrolase activity was studied. As shown in Table I, perfusion of rat hearts for 30 min. with Krebs-Henseleit bicarbonate buffer containing heparin (10 I.U./ml) resulted in a 60% decrease in lipoprotein lipase activity in a 20,000 x g supernatant fraction; a comparable decrease in lipoprotein lipase activity due to perfusion with heparin was also observed in assays with particulate (20,000 x g pellet) subcellular fractions (results not shown). This fraction of total lipoprotein lipase that is heparin-releasable is very similar to that observed by Borensztajn and Robinson (15). In contrast to these results with lipoprotein lipase, perfusion with heparin did not influence neutral triacylglycerol ester hydrolase activity (Table I).

TABLE 1

EFFECT OF HEPARIN

ON LIPASE ACTIVITIES IN PERFUSED HEARTS[a]

Additions to Perfusion Buffer		Enzyme Activity (Units/mg protein)[b]	
		LPL	Neutral TGL
None	(6)[c]	116 ± 12	26 ± 2
Heparin (10 I.U./ml)	(4)	44 ± 8**	25 ± 3

a Hearts were perfused with Krebs-Henseleit bicarbonate buffer in the absence and in the presence of heparin (10 I.U./ml) for 30 min. Lipoprotein lipase (LPL) and neutral triacylglycerol ester hydrolase (neutral TGL) activity was determined in a 20,000 x g supernatant fraction from myocardial homogenates in assays with an ethanolic triolein substrate preparation.
b Activity is presented as the mean ± S.E.M. P values relative to the control (no additions) were determined by student's t test. **, $p<0.01$.
c Number of perfused hearts.

Acid triacylglycerol ester hydrolase activity has also been observed in rat heart (10, 11). Although this acid hydrolase has been assumed to be of lysosomal origin (10, 11), the distribution of lipase activity (determined at pH 5 with an ethanolic triolein substrate) among subcellular fractions obtained by differential centrifugation did not correlate with the distribution of the lysosomal marker enzyme N-acetylglucosaminidase (11). It is possible that this discrepancy may be due to the characteristics of the substrate preparation used to determine acid hydrolase activity, since Severson et al (13) have recently demonstrated that acid triacylglycerol ester hydrolase activity can be measured in a pigeon adipose tissue preparation with a glycerol-dispersed triolein substrate but not with an ethanolic triolein substrate preparation. Consequently, acid and neutral triacylglycerol ester hydrolase activity in a low-speed supernatant fraction from cardiac homogenates was determined with a glycerol-dispersed triolein substrate. The addition of lecithin to the substrate preparation enhanced the expression of acid hydrolase activity but decreased neutral triacylglycerol ester hydrolase activity (Table II); similar results have been obtained previously with the pigeon adipose tissue preparation (13). A comparison of the subcellular distribution of acid and neutral

TABLE II

ASSAY OF RAT HEART
TRIACYLGLYCEROL ESTER HYDROLASE

WITH GLYCEROL-DISPERSED TRIOLEIN SUBSTRATE PREPARATIONS[a]

Substrate Preparation	Enzyme Activity (Units/mg protein)	
	Acid TGL	Neutral TGL
Triolein	8.0 ± 0.2	18.2 ± 1.0
Triolein + lecithin	15.8 ± 0.4	9.2 ± 0.3

a Lipase activity was measured in a 400 x g supernatant fraction from heart homogenates. Results are expressed as the mean ± S.E.M. for six preparations. Acid TGL = acid triacylglycerol ester hydrolase; neutral TGL = neutral triacylglycerol ester hydrolase.

triacylglycerol ester hydrolases with N-acetylglucosaminidase is shown in Figure 1. In agreement with the previous study using an ethanolic triolein substrate (11), the present results indicate that the majority (66%) of the total units of neutral hydrolase activity was localized within the high-speed supernatant fraction. The distribution of neutral hydrolase activity was very similar to that of total protein since the relative specific activity in all subcellular fractions was approximately 1.0. In contrast, the majority (56%) of acid hydrolase activity was particulate, with all particulate fractions showing an enrichment of acid hydrolase relative to the distribution of protein (relative specific activities greater than 1.0). An identical pattern of distribution was observed with the lysosomal marker enzyme N-acetylglucosaminidase for the heavy mitochondrial (M) and crude lysosomal (L) fractions, although a greater fraction (80%) of the total units of N-acetylglucosaminidase were particulate as evidenced by the higher relative specific activity in the microsomal (P) fraction and lower relative specific activity in the soluble (S) fraction (Figure 1).

DISCUSSION

It is generally assumed that lipoprotein lipase in cardiac tissue is present at the endothelial cell surface (16) and within the myocardial

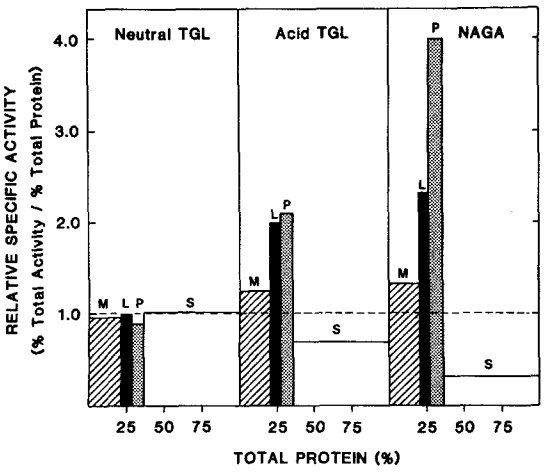

Figure 1. Distribution profiles of enzymes in rat heart subcellular fractions. N-acetylglucosaminidase (NAGA; assayed at pH 4), acid triacylglycerol ester hydrolase (acid TGL; assayed at pH 5 with a glycerol-dispersed triolein substrate preparation containing lecithin), and neutral triacylglycerol ester hydrolase (neutral TGL; assayed at pH 7.5 with a glycerol-dispersed triolein substrate preparation) activites were measured in the following subcellular fractions: 5000 x g pellet (M); 17,000 x g pellet (L); 100,000 x g pellet (P); and 100,000 x g supernatant (S). Results are the mean from six preparations.

cell (17, 18) with the functional role of the lipoprotein lipase in the hydrolysis of lipoprotein-bound triacylglycerols expressed at the capillary endothelium site (15, 19). Perfusion of rat hearts in vitro with buffer containing heparin results in the release of half or more of the lipoprotein lipase activity of the heart into the perfusion medium with a corresponding decrease in the metabolism of lipoprotein triacylglycerols (15). The heparin-releasable lipoprotein lipase is assumed to reflect that portion of the enzyme present in the capillary endothelium and therefore accessible to the heparin in the perfusion medium. In the present investigation, this decrease in lipoprotein lipase as a consequence of heparin perfusion was not accompanied by any change in neutral triacylglycerol ester hydrolase activity (Table 1). Since the removal of the majority of lipoprotein lipase from the heart does not change the activity of the neutral hydrolase, then it can be concluded that the determination of hydrolase activity at pH 7.5 in the absence of serum does not include any contributions from lipoprotein lipase, and that these two hydrolases probably are separate and distinct enzymes.

This conclusion differs from the study of Hülsmann and Stam who determined with heparin-perfusion experiments that no neutral (alkaline) triacylglycerol ester hydrolase apart from lipoprotein lipase was present in cardiac tissue (10).

This discrepancy may reflect methodological differences in the techniques of determining hydrolase activity. In addition, Hülsmann and Stam did not characterize the residual cardiac lipase activity remaining after heparin perfusion except by sensitivity to inhibition by protamine sulfate (10). Acid triacylglycerol ester hydrolase activity has also been observed in cardiac tissue with both ethanolic triolein (11) and glycerol-dispersed triolein substrate preparations containing lecithin (Table II). As discussed by Severson et al (13), glycerol-dispersed substrate preparations are stable and can be stored for several weeks with no appreciable change in assay blanks; this results in hydrolase assays that are both highly reproducible and very sensitive. The addition of lecithin to the glycerol-dispersed triolein substrate preparation resulted in an increase in myocardial acid hydrolase activity; this effect of lecithin likely is a reflection of changes in the "physical state" of the substrate emulsion.

A comparison of the distribution of acid triacylglycerol ester hydrolase activity measured with this glycerol-dispersed triolein substrate preparation containing lecithin with N-acetylglucosaminase provides direct evidence for the proposal that this acid hydrolase is of lysosomal origin. It is not surprising that there were differences in the distribution of the acid hydrolase and N-acetylglucosaminase in the 100,000 x g pellet and supernatant fractions since it is well-established that lysosomes can originate from a variety of cell types within the myocardium (20, 21). The use of high ionic strength buffers in order to obtain an enhanced yield of cardiac lysosomes (22) was not possible since the subsequent presence of KCl in the assay results in an inhibition of acid hydrolase activity as has been observed previously (11). Triacylglycerol ester hydrolase activity has been shown previously to be enriched in lysosomal and microsomal fractions of rat heart homogenates, however lipase activity was measured only at pH 8 (23). An acid lipase, determined with a fluorometric assay using 4-methylumbelliferyl oleate as substrate, has also been show to be enriched in a lysosomal fraction from rat heart (24).

In summary, this investigation has confirmed the presence of both a neutral and an acid lysosomal triacylglycerol ester hydrolase in cardiac tissue. Since rates of hydrolysis of endogenous triacylglycerols in perfused hearts can be increased by catecholamines (1), further studies will have to establish which of these hydrolase activities can be regulated by lipolytic hormones. The observation that chloroquine, a lysosomotropic agent, will decrease rates of lipolysis in perfused hearts and in isolated adipocytes (10) has not been confirmed in subsequent investigations (12, 25).

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