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ON THE DUAL LOCALIZATION OF LIPOPROTEIN LIPASE IN RAT HEART. STUDIES WITH A MODIFIED PERFUSION TECHNIQUE

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SUMMARY

Rat hearts were perfused in vitro using a modified Langendorff technique, allowing the separate collection of coronary- and interstitial effluents. When heparin was added to the perfusion medium lipoprotein lipase was found in the coronary, as well as in the interstitial effluents. The relative amounts of lipase activity in both effluents varied with the feeding conditions of the animals, being high in the coronary effluent during fasting and high in the interstitial effluent during feeding. When glucagon (2.10^{-7} M) was included in the perfusion medium, no differences between fasted and fed animals were obtained. The apparent K_m of the interstitial lipase was lower than that of the lipase found in the coronary effluent. The results are discussed in the light of the localization of lipoprotein lipase in rat hearts in situ.

INTRODUCTION

Serum lipoprotein triglycerides must be hydrolyzed before they can be taken up by the tissue. The hydrolysis is catalyzed by lipoprotein lipase (EC 3.1.1.34) an enzyme (partially) located at the coronary vascular endothelium[1]. The functionally active parts of the enzyme can be measured in heparin-containing perfusates from isolated rat hearts[2-4]. However, in the perfusates not only products from the vascular endothelium are found, but also products from other cell types[5], because of admixture with interstitial fluid. In order to study the relative lipoprotein lipase activities derived from endothelium and the other myocardial cell types, rat hearts were perfused in vitro with a heparin-containing medium and the interstitial fluid and coronary effluent[6] were collected separately.

METHODS AND MATERIALS

Male Wistar rats (200-250 g body weight) were used throughout the study. They were fed ad libitum or fasted overnight as indicated. For the perfusion experiments the animals were anesthetized with intraperitoneally injected sodium pentobarbital (70 mg.kg body weight^{-1}). The hearts were quickly excised, chilled in precooled perfusion buffer until contractions stopped and arranged for retrograde perfusion at a pressure of 10 kPa using a modified Langendorff technique as described by De Deckere and Ten Hoor[6]. In this

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preparation the caval and pulmonary veins are carefully ligated and the pulmonary artery is canulated. It therefore allows the separation of perfusate arising from the myocardial vasculature (Q_{rv}), which leaves the heart by the canulated pulmonary artery, and transmyocardial fluid arising from the interstitial space (Q_i), which drips from the apex of the heart. The mutual contribution of coronary (Q_{rv}) and interstitial effluent (Q_i) to the total flow rate in perfused rat hearts under different experimental conditions is presented in Table I. During control perfusion of hearts from fed or overnight fasted rats Q_i averaged out at 0.24 $ml.min^{-1}.g$ wet wt^{-1} (about 2.5% of total flow rates). The presence of 5 U $heparin.ml^{-1}$, which slightly increased the total flow rate in the hearts, did not influence the percentual contribution of Q_i. Perfusion in the presence of 2.10^{-7} M glucagon significantly increased the total flow rate as well as the percentual contribution of the interstitial washout (Q_i). The perfusion medium was a modified, oxygenated Tyrode buffer[7] supplemented with 11.1 mM glucose. Heparin (5 U/ml) and glucagon (2.10^{-7} M) were dissolved in perfusion buffer. The perfused hearts were electrically stimulated at a rate of 300 $beats.min^{-1}$ by electrodes placed on the right atrium. After a 15 min preperfusion period, in which the canulation and ligation procedures were performed, a control 2 min sample of Q_{rv} and Q_i were collected in cooled tubes (0°C). Subsequent perfusion took place in the presence of heparin ± glucagon and five 2 min samples of Q_{rv} and Q_i were collected. Within 15 min after the start of heparin-perfusion, lipolytic activity was tested in duplicate in 50 µl of each sample, using [^{3}H]triolein as substrate as described in ref. 8. The release of lipolytic activity is expressed in milli units; 1 mU represents the release of 1 nmole fatty acid. min^{-1} from the triglyceride substrate

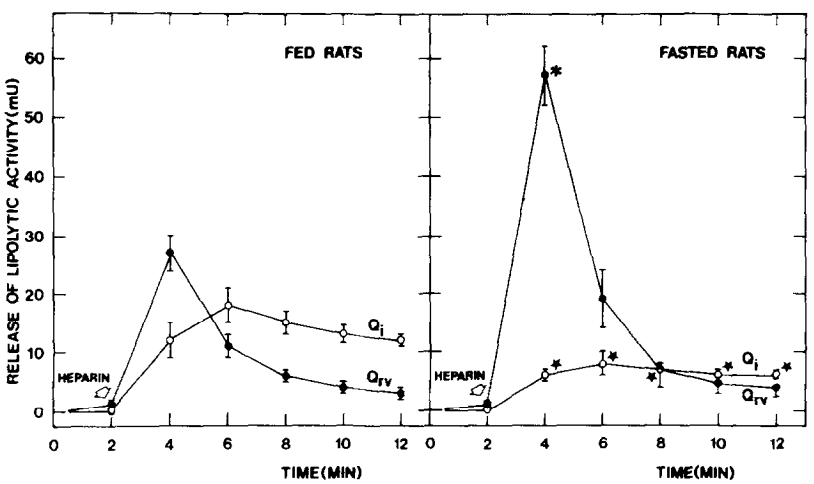

Fig. 1. Release of lipolytic activity from isolated rat hearts by heparin into coronary (Q_{rv}) and interstitial effluents (Q_i). Hearts of normally fed or overnight fasted rats were perfused as described under Methods. After preperfusion with a medium without heparin, heparin was added to the perfusion medium. 2 min fractions of the perfusates were collected during 10 min after the introduction of heparin in the perfusion medium. In the perfusates lipase activities were measured directly. The activities released during 2 min perfusion (mean mU lipase activity ± SEM, n=4) are plotted against perfusion time. At each time point the released activities found with fed rats were compared with the corresponding point of the fasted rats (*: $P<0.025$; ★: $P<0.01$).

All results are presented in mean values ± standard error of the mean (SEM). n represents the number of experiments. Values of P were calculated with Student's t-test (two-tailed). P>0.05 was considered to be not statistically different.

RESULTS

Hearts of normally fed and overnight fasted rats were perfused *in vitro* with a heparin-containing medium and the coronary and interstitial effluents collected separately. Lipase activity was determined in fractions collected during a 2 min perfusion. Both in the coronary- and in the interstitial effluents considerable lipase activity was found (Fig. 1). The lipolytic activity released in coronary effluents from the hearts of fasted rats during the first 2 min of heparin perfusion was significantly higher when compared with hearts of fed animals. However, the activities released in the interstitial effluents were always significantly higher in the fed than in the fasted rats. When glucagon (2.10^{-7} M) was included in the perfusion medium the lipase activities released in the interstitial- and coronary effluents of the hearts from fasted or fed animals were the same (Table II). As demonstrated in Table I, perfusion in the presence of glucagon leads to an increase in both interstitial- and coronary flow rates. This may result in a better washout of the enzyme from the tissue. Moreover, *in vitro* perfusion of rat hearts without heparin but in the presence of glucagon during 5 min did not result in significant changes in the total lipase activity in the hearts (415±49 mU (n=4) in hearts perfused without glucagon and 440±77 mU (n=4) in hearts perfused with glucagon). The lipase activities released into the coronary and the interstitial effluents exhibited characteristics of lipoprotein lipase. The activities were largely

TABLE I

CONTRIBUTION OF CORONARY (Q_{rv}) AND INTERSTITIAL EFFLUENTS (Q_i) TO TOTAL FLOW RATES IN PERFUSED RAT HEARTS

Rat	Heparin (5 mU/ml)	Glucagon (2.10^{-7} M)	n	Flow rate (ml.min^{-1}.g wet wt^{-1})	% in Q_{rv}	% in Q_i
Fed	-	-	19	9.3±0.5	97.5±0.3	2.5±0.3
Fasted	-	-	10	10.4±0.9	97.6±0.3	2.4±0.2
Fed	+	-	5	11.7±1.5	97.4±0.2	2.6±0.2
Fasted	+	-	4	12.0±1.1	97.3±0.4	2.7±0.4
Fed	+	+	9	16.0±0.8*	96.0±0.6	4.0±0.2*
Fasted	+	+	5	16.1±0.9*	95.7±0.6	4.3±0.4*

*P<0.05 *vs* fed or fasted + heparin

Rat hearts were perfused as described under Methods. The volumes of the perfusates in Q_{rv} or Q_i were measured and expressed in ml.min^{-1}.g wet heart weight^{-1}.

TABLE II

THE EFFECT OF GLUCAGON ON THE HEPARIN-INDUCED RELEASE OF LIPOLYTIC ACTIVITY IN CORONARY- (Q_{rv}) AND INTERSTITIAL EFFLUENTS (Q_i) OF PERFUSED RAT HEARTS

Condition	n	Total released lipolytic activity (mU/heart ± SEM)		
		Q_{rv}	Q_i	Total
Fasted	4	92±19	33±6	125±23
		P<0.05	P<0.05	NS
Fed	4	50±4	NS* 68±12	P<0.005 118±15
				P<0.05
Fasted + glucagon	4	119±5	74±12	193±15
		P<0.005	NS	NS
Fed + glucagon	3	139±17	91±9	230±19
				P<0.01

*NS: not statistically significant

Rat hearts were perfused as described in the legends of Fig. 1. When indicated, together with heparin, glucagon (2.10^{-7} M) was added to the perfusion medium. The lipase activities released during 10 min perfusion in the coronary- and interstitial effluents were determined. Total activities were obtained by addition of the values in Q_{rv} and Q_i of the separate experiments.

dependent on the presence of apolipoprotein C_{II} and were inhibited by 1 M NaCl (Table III). The apparent K_m's of both activities, however, were different. The lowest K_m was found in the interstitial effluent (Table IV).

DISCUSSION

Lipoprotein lipase is found in cultured neonatal rat heart cells[9-11] and myocytes isolated from adult rat hearts[12,13]. Part of the lipase in rat hearts is located at the vascular endothelium, where it may exert its function in the hydrolysis of serum lipoprotein triglycerides[1-4]. The distribution of

TABLE III

CHARACTERISTICS OF THE LIPASE ACTIVITY RELEASED INTO CORONARY (Q_{rv}) AND INTERSTITIAL EFFLUENTS (Q_i) FROM RAT HEARTS

Condition of the rats	Lipase activity (mU/ml perfusate)					
	Coronary effluent (Q_{rv})			Interstitial effluent (Q_i)		
	-apo C_{II}	+apo C_{II}	+1 M NaCl	-apo C_{II}	+apo C_{II}	+1 mM NaCl
Fed	1.1	3.2	0.6	6.0	17.6	3.9
Fasted	1.1	3.4	0.7	3.0	10.1	1.2

The hearts of 2 normally fed and 2 overnight fasted rats were perfused with a heparin-containing medium as described in the legends of Fig. 1. The perfusates were collected during 10 min. Lipase activities were estimated as described in ref. 8. However, serum in the substrates was replaced by 0.9% NaCl. Where indicated 4 µg of purified apolipoprotein C_{II} was added to the serum-free substrate. In some instances NaCl in a final concentration of 1 M was added to the incubations containing apolipoprotein C_{II}. The means of the results obtained with the different rats are shown.

TABLE IV

APPARENT K_m VALUES OF LIPOPROTEIN LIPASE(S) OF CORONARY VASCULAR (Q_{rv}) AND INTERSTITIAL (Q_i) SPACES

Apparent K_m (mM)	coronary effluent Q_{rv})	2.73±0.45
	interstitial effluent (Q_i)	1.04±0.27*

*P<0.05

The coronary effluents (Q_{rv}) of normally fed rats were obtained during 1 min perfusion after the introduction of heparin. The interstitial effluents (Q_i) were collected between 5 and 7 min. The means ± SEM of 3 separate experiments are shown.

lipase activity between endothelium and muscle cells is not known. Chohan and Cryer[13] suggested that an important part of the total lipoprotein lipase of rat hearts may be associated with the muscle cells. On the other hand, Henson et al.[10] and Chajek et al.[11] showed that the lipase may be synthesized in different cell types. Recently, De Deckere and Ten Hoor[6] described a technique by which during in vitro heart perfusion coronary- and interstitial effluents can be collected separately. The coronary effluent contains products mainly derived from the vascular endothelium, while the interstitial effluent mainly contains products from other cell types (e.g. cardiac myocytes)[5,6]. Using this technique, we found that lipoprotein lipase in heparin-containing perfusates of rat hearts may be derived from the endothelium as well as from other cell types. The activity derived from either cell type is largely dependent on the feeding condition of the animals. During fasting and feeding a redistribution of enzyme activity between the coronary (endothelial) and interstitial (myocytal) spaces occurs. Hülsmann and Stam[14] had shown that norepinephrine enhances the heparin-releasability of rat heart lipoprotein lipase. This was recently confirmed by Simpson[15] who found that glucagon had a similar effect. We have found that glucagon enhances the heparin-releasable activity in the interstitial effluent from the fasted and in the coronary effluent of the fed animals. Therefore, in the presence of glucagon the activities released in coronary- and interstitial effluents of fed and fasted animals are similar. This suggests that the differences in activities in the absence of glucagon are due to differences in the heparin-releasability rather than to differences in enzyme content. Whether the increase of heparin-releasable activities by glucagon depends on activation of inactive enzyme, a better washout of the enzyme or stabilization of the enzyme remains to be established. The lipase activities derived from the interstitial- and coronary space may be catalyzed by identical or different enzymes. The differences in apparent K_m's support the latter possibility. Different

forms of lipoprotein lipase have been found in rat heart earlier[16]. To what extent the activities released from both compartments are catalyzed by the same enzyme and to what extent both activities are functionally active in situ, is under present investigation. The perfusion technique used in this study may help to answer these questions.

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