

## DEMONSTRATION OF A HEPARIN-RELEASABLE LIVER-LIPASE-LIKE ACTIVITY IN RAT ADRENALS

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### 1. Introduction

The liver contains an acylglycerol hydrolase activity (liver-lipase) which is releasable by heparin perfusion [1]. Recent work has indicated that this lipase may be involved in the cellular uptake of high density lipoprotein phospholipids and cholesterol from the plasma [2,3]. It has been suggested that high density lipoprotein phospholipids can be hydrolyzed by the enzyme, thereby enabling the transfer of high density lipoprotein cholesterol to the liver [2–4]. The question then arises whether such a mechanism would be only operative in the liver or also in other high density lipoprotein lipid-utilizing organs. Therefore, we studied whether the adrenal gland, an organ able to take up and utilize high density lipoprotein cholesterol as a substrate for steroid hormone synthesis [5–7], also contains an enzyme similar to liver-lipase. A neutral lipase activity could be extracted from rat adrenals by a heparin-containing medium. This activity was not influenced by 1 M NaCl and slightly (30%) inhibited by serum. The activity was strongly (86%) inhibited by an antibody raised against liver-lipase. The lipase could be bound to Sepharose–heparin and eluted by a salt gradient, the pattern of which was identical to that of liver-lipase. It is concluded that adrenals contain a lipase similar to the heparin-releasable lipase of rat liver. In the adrenals of female rats the activity of the lipase (per gram tissue wet weight) was equal to the activity found in the livers ( $226 \pm 14$  mU vs  $224 \pm 13$  mU, respectively). In rats with a corticotrophin- and prolactin-secreting pituitary tumor the activity in the adrenals (per gram wet weight) was significantly higher than in the liver ( $221 \pm 35$  mU vs  $160 \pm 13$  mU).

### 2. Materials and methods

Normally fed rats were used. The rats were of the Wistar strain unless stated otherwise. Adrenals and livers were removed from the rats under ether narcosis and immediately cooled on ice. Adrenals were trimmed of fat and weighed. Homogenates of the adrenals and of 50–100 mg liver were made in cold 0.15 M NaCl containing 5 IU heparin/ml (Thromboliquine, Organon, Oss), using a PT 10 Polytron tissue homogenizer (10 s). The homogenates were centrifuged in plastic reaction vessels in an Eppendorf 3200 centrifuge at 4°C.

Lipase activities were measured as the release of free fatty acids from a glycerol [<sup>3</sup>H]trioleate emulsion stabilized with gum arabic, at pH 8.5, as in [8]. Lipase activities are expressed in mU (nmol free fatty acids released/min). In the experiments on the pH dependency of adrenal lipase activity the assays were carried out in the same way except that the following buffers (0.1 M) were used: sodium acetate pH 4.0, 4.5, 5.0; sodium phosphate pH 5.8, 6.4, 6.8; Tris–HCl pH 8.0, 8.9; glycine–NaOH pH 9.8 (cf. [9]).

Antibodies were raised in rabbits against liver-lipase purified from heparin-containing rat liver perfusates [10]. From the antiserum the  $\gamma$ -globulin fraction was isolated by precipitation with  $(\text{NH}_4)_2\text{SO}_4$  and protein A affinity chromatography [2]. The  $\gamma$ -globulins were taken up in 0.15 M NaCl to 4 mg protein/ml (= anti-liver-lipase). 250  $\mu$ l antibody solution inhibits 190 mU liver-lipase completely and does not affect lipoprotein lipase [2]. In control experiments the  $\gamma$ -globulin fraction of an antiserum raised in rabbits against non-heparin-releasable monoglyceridase from rat liver [11] was used (control  $\gamma$ -globulins).

Female bastard rats (Buffalo  $\times$  Wag/Rij) of  $\sim$ 200 g

received a tumor from the corticotrophin/prolactin secreting tumor 7315a into the scapular region 4 weeks before the animals were studied [12].

### 3. Results

Homogenates of rat adrenals were found to exhibit lipase activities at acid and neutral (alkaline) pH (fig.1). After centrifugation of the heparin-containing homogenates almost no lipase activity was found at acid pH, while ~75% of the neutral lipase activity was recovered in the supernatant (fig.1). In 4 different expt  $73 \pm 4\%$  (mean  $\pm$  SD) of the total homogenate activity was found in the supernatants when measured under standard conditions (pH 8.5). The lipase activity in the supernatants was inhibited for 30% by the addition of 10% fasted rat serum to the assay mixture and not affected by 1 M NaCl (table 1). An antibody against the lipase, releasable from rat livers by heparin, inhib-

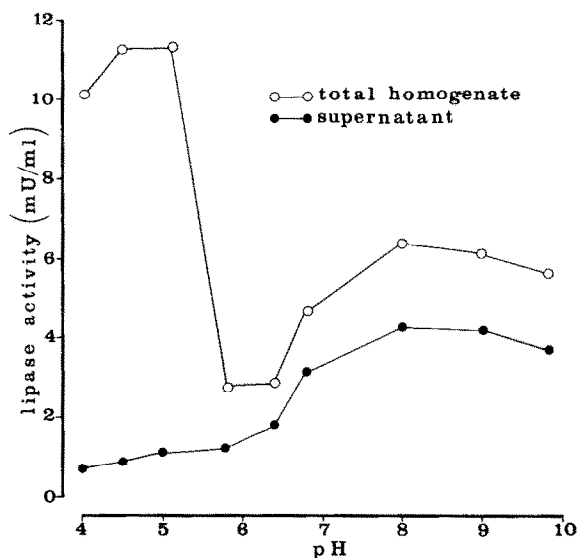


Fig.1. pH profile of lipase activity in homogenates and supernatants of rat adrenals. Adrenals of 2 rats were homogenized in 4 ml 0.15 M NaCl containing 5 IU heparin/ml. Part of the homogenate was sonified for 5 s at 0°C with a Branson Sonifier at 21 kHz. The other part of the homogenates was centrifuged for 5 min at 8000  $\times$  g and the supernatant was sonified. Lipase activities were measured in duplicate in 50  $\mu$ l homogenate and supernatant. The data shown represent a typical example out of 3 separate expt. The activities are expressed in mU/ml homogenate ( $\circ$ — $\circ$ ) or supernatant ( $\bullet$ — $\bullet$ ).

Table 1  
Effects of serum and high salt concentration on the neutral lipase activity in rat adrenals

Additions	Lipase activity (% control)
None	100
Rat serum, 10%	70 $\pm$ 5
NaCl, 1.0 M	109 $\pm$ 8

The adrenals of 1 rat were homogenized in 2 ml 0.15 M NaCl containing 5 U heparin/ml. The homogenate was centrifuged for 5 min at 8000  $\times$  g. Lipase activity was measured in the supernatant in the presence or absence of 10% fasted rat serum, with or without 1.0 M NaCl (final conc.). The activity measured without additions was taken as 100%. The mean values  $\pm$  SD of 3 separate expt are given

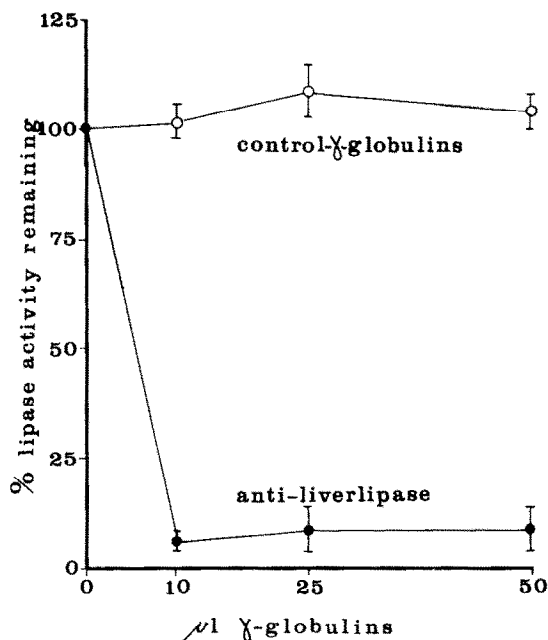


Fig.2. Inhibition of adrenal lipase by anti-liver-lipase. Adrenal homogenates were prepared as under section 2 in heparin-containing saline (2 adrenals/4 ml medium) and centrifuged for 5 min at 8000  $\times$  g. Aliquots (50  $\mu$ l) of the supernatants were mixed with different amounts of anti-liver-lipase ( $\bullet$ — $\bullet$ ) or control  $\gamma$ -globulins ( $\circ$ — $\circ$ ), both containing 4 mg protein/ml (see section 2). The samples were incubated for 5 min at 37°C followed by 30 min at 0°C, then lipase activities were measured in the samples. The activities were expressed as the % of the activities measured without added globulins ( $3.7 \pm 0.5$  mU/ml supernatant = 100%). The lipase activity in the supernatant was not affected by preincubation in the absence of added  $\gamma$ -globulins (not shown). The means of 3 separate expt  $\pm$  SD are given.

Table 2  
Lipase activities in the adrenals and livers of control and pituitary tumor-bearing rats

No. of rats	Antibody-sensitive lipase activity in				Liver		
	Adrenals						
	mU/g wet wt	mU/mg protein	mU/2 adrenals	mU/g wet wt	mU/mg protein	mU/liver	
Control rats	3	193 ± 25	32.8 ± 4.1	11.6 ± 2.0	236 ± 1.0	21.9 ± 1.3	1861 ± 34
		n.s.	n.s.	p < 0.01	p < 0.005	p < 0.05	p < 0.01
Tumor rats	3	221 ± 35	36.6 ± 5.1	27.7 ± 2.4	160 ± 13	15.3 ± 1.6	1296 ± 101
Control rats (non age-matched)	14	226 ± 14	n.d.	n.d.	224 ± 13	n.d.	n.d.

The statistical significance of the differences found between control and tumor rats was calculated using Student's *t*-test (unpaired, 2-tailed); n.s., non-significant; n.d., not determined

A pituitary-tumor was induced in 3 female rats. After 4 weeks the rats were killed, together with 3 control rats of the same age. Adrenals and livers were removed. The adrenals and ~50 mg liver of each rat were homogenized in 1 ml 0.9% NaCl containing 5 IU heparin. The tissue homogenates were centrifuged for 5 min at 8000 × *g*. Then 50 µl of the supernatants were incubated with or without 10 µl anti-liver-lipase for 5 min at 37°C and then 30 min at 0°C. Lipase activities were determined as in [8] in duplicate. In all samples the antibody inhibited 80–87% of the total lipase activity. The activities inhibited by the antibody were calculated by subtraction of the remaining activities from the total lipase activities. The means of the activities inhibited by the antiserum obtained in the age-matched control and tumor rats are given ± SEM, as well as the lipase activities inhibited by the antibody obtained in the adrenals and liver of female non-age-matched controls

ited the solubilized lipase activity for 90–95%, while control antibodies (raised against non-heparin-releasable rat liver monoglyceridase) had no effect (fig.2). The inhibition of the solubilized lipase activity by 10  $\mu$ l anti-liver-lipase was determined in a large number ( $n = 18$ ) of experiments. The percentage inhibition varied from 75–95% (mean  $\pm$  SD  $86 \pm 8$ ). The residual activity in the pellet of the homogenates was inhibited by the antibody for  $56 \pm 3\%$  ( $n = 4$ ).

The lipase activity of an extract of a defatted adrenal preparation could be bound to a Sepharose–heparin column. The activity was eluted from the column at the same [NaCl] (0.7 M) as the lipase activity derived from rat liver (fig.3). These results indicate that adrenals contain a lipase activity that resembles or may even be identical with the heparin-releasable lipase from rat liver. On a tissue wet weight base the activities of adrenal- and liver-lipase in female rats were found to be equal (table 2). In rats in which a corticotrophin- and prolactin-producing tumor had been induced, the lipase activity in the liver was significantly lowered (table 2). Therefore, in the tumor-

bearing rats the lipase activity in the adrenals (per gram tissue) is significantly higher than that in the liver. The total lipase activity in the adrenals of the tumor-bearing rats is significantly enhanced when compared to that in the adrenals of control rats, since the first are enlarged (hyperplastic) ( $138 \pm 35$  mg vs  $59 \pm 6$  mg, means  $\pm$  SD,  $n = 3$ ).

#### 4. Discussion

After injection of heparin into several species at least 2 different lipases are found in the plasma [1,13]. One of the lipases is lipoprotein lipase (EC 3.1.1.34). The other is an acylglycerol hydrolase that is immunologically different from lipoprotein lipase [13]. This lipase is found in liver, from which it can be released by *in vitro* perfusion with a heparin-containing medium [4,14]. This activity has not been found in other tissues such as heart, adipose tissue or lung [13]. That the liver is the main source of this post-heparin lipase in rats is illustrated by the loss of post-heparin serum activity after hepatectomy [1,13]. Therefore, this lipase is called liver-, hepatic- or hepatic endothelial-lipase [3].

This paper shows that rat adrenals contain a lipase activity different from acid lipase. This neutral lipase is partly inhibited by serum, as was described for liver-lipase before [15] and not inhibited by 1 M NaCl and therefore is different from lipoprotein lipase. Also the lipase is largely inhibited by an antiserum raised against liver-lipase, purified from heparin-containing liver perfusates. It behaves on a Sepharose–heparin column identical to lipase from rat liver. Based on these data we conclude that adrenals contain, besides acid lipase, a lipase similar to liver-lipase. Preliminary experiments, using anti-human liver-lipase and a female human adrenal, showed that also in man adrenals may contain liver-lipase-like activity. The activity per gram wet weight was  $\sim 25\%$  of that observed in rats (52 mU vs 226 mU). The contribution of the adrenals to the total post-heparin plasma activity will be very low because of the relatively low adrenal weight. Still, the lipase may exert locally an important action, as the activity per gram tissue wet weight is equal in the adrenals and in the liver. The function of this lipase type in lipid metabolism is not established yet. Injection of an antibody against the liver-lipase results in an increased serum high density lipoprotein–cholesterol level [2,3]. In patients on chronic hemodialysis a

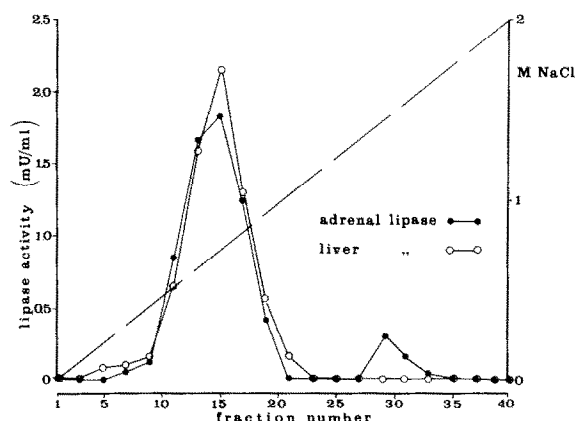


Fig.3. Elution of lipase activity of rat adrenals and liver from a Sepharose–heparin column by NaCl. The adrenals of 14 female rats were defatted with acetone and ether. The powder (130 mg) was homogenized in 6 ml 10 mM phosphate (+5% glycerol) buffer (pH 7.0). The homogenate was centrifuged for 2 min at  $8000 \times g_m$ . Supernatant (4.9 ml, containing 23.6 mU lipase activity) was applied to a Sepharose–heparin column ( $0.9 \times 3$  cm). The column was washed with 40 ml phosphate buffer. Then a linear salt gradient (40 ml) from 0–2 M NaCl was applied to the column. Fractions of 1 ml were collected and lipase activity determined in 100  $\mu$ l of the fractions (●—●). The same experiment was carried out with 130 mg acetone powder of one of the rat livers used (○—○).

negative correlation between high density lipoprotein—cholesterol and postheparin plasma liver-lipase activity can be shown (L. Verschoor, et al. in preparation). Following oestrogen treatment, liver-lipase is lowered [16] and high density cholesterol levels are elevated [17]. In women, compared to men, lower lipase activities [18] and higher density lipoprotein—cholesterol concentrations [19] are found. These data point to a role of the liver-lipase in the clearing of high density lipoprotein—cholesterol. It is possible that dependent on the activity of the lipase in situ, more or less high density lipoprotein—cholesterol can be taken up by the tissue containing this enzyme. In this way liver-lipase (-like) activities in high density lipoprotein—cholesterol utilizing organs (liver, adrenals and perhaps other steroid hormone producing organs) may help to channel high density lipoprotein—cholesterol to these organs. Such a role of the enzyme would be analogous to the directive role of lipoprotein lipase in the uptake of serum triglycerides in different organs [20]. In this respect it is of interest to note that under conditions of high corticosteroid synthesis (the pituitary tumor-bearing rats) the total activity in the adrenals is significantly elevated, while that in liver is significantly decreased. The relative importance of such a system, compared to other regulatory processes [5–7], for the uptake of high density lipoprotein—cholesterol has to be established.

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