Rat liver contains a limited number of binding sites for hepatic lipase

Kees SCHOONDERWOERD,* Adrie J. M. VERHOEVEN and Hans JANSEN
Department of Biochemistry, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

The binding of hepatic lipase to rat liver was studied in an *ex vivo* perfusion model. The livers were perfused with media containing partially purified rat hepatic lipase or bovine milk lipoprotein lipase. The activity of the enzymes was determined in the perfusion media before and after passage through the liver. During perfusion with a hepatic-lipase-containing medium the lipase activity in the medium did not change, indicating that there was no net binding of lipase by the liver. In contrast, more than 80% of the lipoprotein lipase was removed from the medium. This lipoprotein lipase activity could be recovered into the perfusion medium completely by heparin perfusion of the liver. If livers, first depleted of hepatic lipase by heparin, were subsequent perfused with a hepatic-lipase-containing medium, 90 ± 24 m-units of the lipase activity was bound per g of liver (up to 1000 m-units/total liver). However, heparin treatment of the liver decreases the ability of the liver to re-bind hepatic lipase by 80%. Perfusion of rat livers with 0.3 M NaCl released 60% of the lipase activity into the medium. Upon subsequent perfusion of these livers with hepatic-lipase-containing media, 541 ± 164 m-units of hepatic lipase could be bound per g of liver (up to 5000 m-units/total liver). The binding of hepatic lipase was also studied in livers of corticotropin (ACTH)-pre-treated rats. In these rats also, hepatic lipase bound only to livers which had been pre-perfused with heparin or 0.3 M NaCl. After heparin pre-perfusion, 88 ± 12 m-units of hepatic lipase could be bound per g of liver, similar to that with livers of control rats not treated with ACTH. After prior salt perfusion, however, the capacity of the livers of ACTH-pre-treated rats to bind hepatic lipase was 212 ± 60 m-units/g of liver. This is less than in livers of control rats (541 ± 164 m-units/g of liver). These results indicate that in rat liver the binding of hepatic lipase is heterogeneous in character and consists of heparin-resistant and heparin-sensitive components. The hepatic-lipase binding capacity of the liver is saturable and fully utilized under various conditions. The heparin-sensitive binding capacity is lowered in ACTH-treated rats, whereas the heparin-resistant binding is unaffected. We postulate that the functional hepatic lipase activity can be regulated by changes in the binding capacity of the liver.

INTRODUCTION

Heparin releases two lipases into the bloodstream, lipoprotein lipase and hepatic lipase [1–4]. Lipoprotein lipase originates from extra-hepatic tissues and is involved in the metabolism of triacylglycerol-rich lipoproteins. Hepatic lipase is present in the liver and is believed to play a role in the metabolism of high-density lipoproteins, intermediate-density lipoproteins and chylo-micron remnants [5–9]. Both lipoprotein lipase and hepatic lipase are synthesized and secreted by parenchymal cells and are bound specifically to the endothelial-cell lining [10–15]. It is generally assumed that both hepatic lipase and lipoprotein lipase are bound to heparan sulphates at the endothelium [15–18]. A heparan sulphate proteoglycan and a heparin-releasable lipoprotein-lipase-binding protein (HRP) have been identified as the binding sites for lipoprotein lipase in bovine aortic endothelial cells [19,20]. *In vitro*, a saturable binding of lipoprotein lipase is observed with bovine aortic endothelial cells, and it has been suggested that the binding capacity for lipoprotein lipase is determined by the number of heparan sulphate molecules in the extracellular matrix [15]. However, *in situ* no saturation of lipoprotein lipase binding was observed in perfused rat hearts [21], indicating that, at least in hearts, the binding sites for lipoprotein lipase are not fully occupied under normal conditions. Lipoprotein lipase can also be bound to the liver in a non-saturable manner, and binding of lipoprotein lipase to the liver is considered as part of the degradation route of lipoprotein lipase [22–24]. Additionally, binding of lipoprotein lipase to the liver may play a role in the uptake of lipoproteins by the liver [25–28].

Less is known about the binding of extracellularly located hepatic lipase in the liver. Heparan sulphates appear also to be involved in the binding of hepatic lipase to HepG2 cells [29]. Hepatic lipase binds to isolated non-parenchymal liver cells in a saturable manner [11]. Previously we have shown that the hepatic lipase activity is greatly lowered in hypercorticism [12]. Part of the lowering may be due to decreased synthesis and secretion of the enzyme by the parenchymal liver cells, as studied in corticotropin (ACTH)-treated animals. Besides the decreased synthesis, also the maximal binding capacity of the non-parenchymal cells for hepatic lipase is decreased after prior treatment of the animals with ACTH [12]. This suggests that the binding capacity of liver endothelium for hepatic lipase may be limited. Almost all hepatic lipase is extracellularly located, implying that the number of hepatic-lipase binding sites determines the functionally expressed activity of the lipase in the liver. However, a decrease in maximal binding capacity hardly affects the expressed activity if only a few binding sites are occupied under most conditions. Therefore, we studied the availability of hepatic-lipase binding sites in livers of control and ACTH-pre-treated rats *in situ*.

MATERIALS AND METHODS

Animals

Normally fed male Wistar rats (250–300 g) were used. They were housed under controlled conditions: temperature 20–22 °C, light on 07:00–19:00 h. Hypercorticism was induced by treatment with a synthetic ACTH analogue (Synacthen-depot, 2 mg/ml) [12,30]. Synacthen was administered during 3 days sub-

Abbreviations used: ACTH, corticotropin; KRB, Krebs–Ringer bicarbonate buffer.

* To whom all correspondence and reprint requests should be addressed.

cutaneously (0.2 mg/kg body weight). Synacthen-treated rats are indicated as 'ACTH-rats'.

Isolation of lipoprotein lipase and hepatic lipase

Lipoprotein lipase was purified from bovine milk as described by Tajima and co-workers [31]. Lipoprotein lipase was first bound to Sepharose–heparin and eluted with 1.5 M NaCl/10 mM sodium phosphate, pH 6.8. Fractions containing lipoprotein lipase activity were pooled and diluted to 0.5 M NaCl. After binding to Sepharose–heparin again, lipoprotein lipase activity was eluted with a linear salt gradient of 1–2 M NaCl/10 mM sodium phosphate, pH 6.8. The eluted lipoprotein lipase activity was loaded on to a hydroxyapatite column equilibrated with 10 mM sodium phosphate, pH 6.8. After elution with 0.3 M sodium phosphate/0.5 M NaCl/20% glycerol, pH 6.8, the enzyme was precipitated by dialysis against 3.6 M $(NH_4)_2SO_4$. The precipitate was collected by centrifugation, and the purified lipoprotein lipase was suspended in 20 mM sodium phosphate/50% glycerol, pH 6.8 (specific activity 44150 m-units/mg of protein).

Hepatic lipase was isolated from heparin-containing rat liver perfusates [12]. Rat livers were perfused through the portal vein at 37 °C with a Krebs–Ringer bicarbonate buffer (KRB: 140 mM NaCl, 5.4 mM KCl, 0.8 mM Na_2HPO_4, 2.5 mM $CaCl_2$, 8.1 mM $MgSO_4$, 25 mM $NaHCO_3$, 6.0 mM glucose, pH 7.4) containing 1% BSA at a flow rate of 3–4 ml/min per g of liver. After 10 min pre-perfusion, the perfusion was continued for 5 min with the same medium to which 10% glycerol and 5 units/ml heparin were added. The perfusates were collected on ice and applied to a Sepharose–heparin column (1.5 cm × 9 cm; flow rate 35 ml/h, 4 °C). The column was washed with 3 column volumes of 0.2 M NaCl/10 mM sodium phosphate/10% glycerol, pH 7.4. Lipase activity was eluted with the same buffer containing 1 M NaCl and 1% BSA; 1 ml fractions were collected. The five peak fractions were combined and the buffer was changed to KRB containing 1% BSA by gel-filtration using PD10 columns.

Liver perfusion

Livers were perfused *in situ* as described by Seglen [32]. The animals were anaesthetized with diethyl ether. The portal vein was cannulated and the perfusion was started immediately with KRB at a flow rate of 3–4 ml/min per g of liver. The vena cava superior was cannulated just above the diaphragm and the vena cava inferior was ligated just above the right renal vein. After a 10 min wash-out period, the perfusion medium was changed to KRB containing 1% BSA and perfusion was continued at 0–4 °C; 5 min later hepatic lipase or lipoprotein lipase was added to the medium. The perfusion was continued in a recirculating manner during 10 min with a total volume of 50 ml. At the indicated time points, samples from the medium were taken for the determination of hepatic lipase or lipoprotein lipase. After the reperfusion period the liver was flushed for 5 min with KRB containing 1% BSA to remove unbound enzyme. Finally, heparin (5 units/ml) was added to the buffer, and the heparin perfusate was collected on ice. In some experiments livers, after the 10 min wash-out period, were perfused with 0.3 M NaCl by adding 0.16 M NaCl to the perfusion medium. The perfusion with 0.3 M NaCl during 10 min was always followed by perfusion for 5 min with 0.22 M NaCl and 5 min with KRB, successively. Binding of hepatic lipase to these 0.3 M NaCl-pre-perfused livers was performed as described above. It was found that the salt perfusion, provided that the salt concentration was gradually lowered to a physiological concentration, caused no functional damage to the liver, as assessed by measurement of lactate dehydrogenase activity in the medium (results not shown).

Antibodies

Hepatic lipase, purified by the method of Jensen and Bensadoun [33], was used to immunize a goat as described elsewhere [34]. The IgG fraction was purified from plasma by two successive precipitations with 50%-satd. $(NH_4)_2SO_4$ and 17%-satd. Na_2SO_4 respectively, followed by extensive dialysis against PBS. Final protein concentration was 20 mg/ml; 1 ml of this preparation inhibits 3500 m-units of hepatic lipase activity in a liver perfusate. IgGs isolated similarly from the serum of a non-immunized goat did not affect hepatic lipase activity. In a Western blot of a heparin-containing liver perfusate, a single band of 58 kDa, corresponding to hepatic lipase, was recognized by the specific IgG preparation, but not by the control IgGs (results not shown).

Monoclonal antibodies against hepatic lipase have been described previously [34]. A mixture of hybridoma supernatants of the five different clones was used in the present study. The titre of this preparation was about 20 m-units/ml.

Hepatic lipase and lipoprotein lipase activity assays

Lipoprotein lipase activity was measured by using a glycerol-stabilized glycerol [9,10(n-^{3}H)]trioleate suspension as described by Nilsson-Ehle and Schotz [35]. To avoid any possible contamination with hepatic lipase activity, samples were preincubated with an anti-(hepatic lipase) specific polyclonal antibody [34]. The samples were mixed with anti-(hepatic lipase) specific IgG (1/1, v/v) and preincubated for 5 min at 25 °C, followed by 25 min on ice, before the lipase assay was carried out.

Hepatic lipase activity was determined in the perfusion media and in post-nuclear supernatants of a 2.5% (w/v) liver homogenate in PBS containing 5 units/ml heparin (15 s, Polytron setting 4). Hepatic lipase was measured during a 30 min incubation at 30 °C by using an artificial glycerol [9,10(n-^{3}H)]-trioleate emulsion in gum arabic at 1.0 M NaCl, pH 8.5 [36]. Lipoprotein lipase activity is not detectable under these conditions. All the lipase activity measured could be completely suppressed by anti-hepatic lipase antibody (results not shown).

E.l.i.s.a. for rat hepatic lipase

The amount of hepatic lipase protein was determined by a solid-phase e.l.i.s.a. [37]. Polystyrene microtitre plates (96 wells) were coated with the goat anti-(hepatic lipase) IgG. After blocking with gelatin, the wells were successively incubated with sample, monoclonal antibodies (diluted 5-fold) and alkaline phosphatase-conjugated goat anti-mouse IgG (diluted 1:500) in the presence of 1 M NaCl, 0.1% Tween 20 and 0.5% BSA to decrease non-specific binding. Finally, alkaline phosphatase activity was measured with *p*-nitrophenol phosphate as the substrate in a Titertek EIA analyser. Absorbances at 405 nm were read against a standard curve for partially purified hepatic lipase.

Materials

All chemicals used were of analytical grade. Heparin (Thromboliquine) was purchased from Organon-Teknika (Boxtel, The Netherlands) and BSA from Sigma (St. Louis, MO, U.S.A.). Glycerol [9,10(n-^{3}H)]trioleate (sp. radioactivity 1 Ci/mmol) was obtained from Amersham International,

Amersham, Bucks., U.K. Synacthen was from Ciba–Geigy A. G., Basel, Switzerland. Alkaline phosphatase-conjugated anti-mouse IgG was purchased from Tago, Burlingame, CA, U.S.A.

RESULTS

Liver perfusion with lipase-containing media

The livers of normally fed rats were perfused with a medium containing either partially purified hepatic lipase or lipoprotein lipase. During a 10 min recirculating perfusion with various amounts of hepatic lipase (2000–7000 m-units), the lipase activity and hepatic lipase mass in the medium did not change (Figure 1). Similar results (not shown) were obtained during perfusion at 4 °C and 37 °C. The lipase activity in the livers also was essentially unaffected (without perfusion 820 ± 100 m-units/g of liver, after perfusion 780 ± 75 m-units/g of liver; $n = 3$). In contrast, during perfusion with lipoprotein lipase, the lipoprotein lipase activity in the medium fell within 5 min to 30 % of the initial value. The lipoprotein lipase activity lost from the medium during the perfusion was completely recovered by subsequent heparin perfusion (Figure 2). The amount of lipoprotein lipase which bound to the liver increased linearly with the amount of lipoprotein lipase present in the perfusion medium up to 1500 m-units (Figure 3). In control experiments, the perfusion protocol was carried out without a liver. Under these conditions no change in either hepatic lipase or lipoprotein lipase activity in the medium was observed (results not shown).

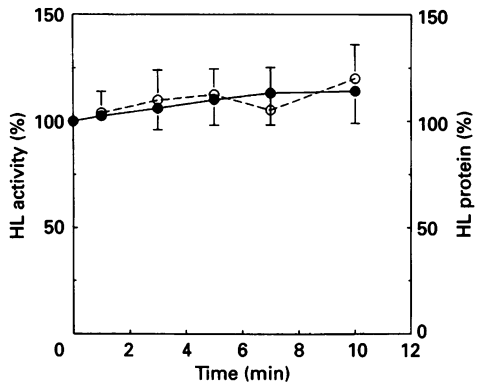

Figure 1 Binding of hepatic lipase (HL) to perfused rat liver

Rat livers were perfused at 4 °C with various amounts of hepatic lipase (up to 7000 m-units). At different time intervals samples were withdrawn and lipase activity (●) or amount of hepatic lipase protein (○) was determined in the perfusate. The results are expressed as % of the initial lipase activity present in the perfusate (100%). The results of three different experiments are given (means ± S.D.).

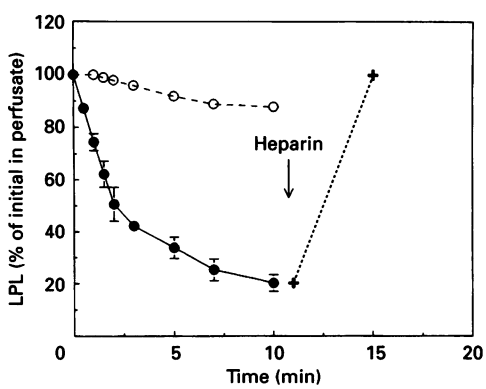

Figure 2 Binding of lipoprotein lipase (LPL) to perfused rat liver

Lipoprotein lipase was perfused through rat liver in recirculating mode (●——●) or without rat liver (○----○) at 4 °C with a medium containing lipoprotein lipase. Lipoprotein lipase activity was measured in the recirculating perfusion media. After the recirculating perfusion the livers were perfused one way with KRB, followed by perfusion with a heparin-containing medium (5 units/ml) during 5 min. Lipoprotein lipase activity was measured in the perfusate at the end of the recirculating perfusion and at the end of the heparin perfusion (+ · · +). The initial lipase activity present in the perfusate is taken as 100% (200–1500 m-units). Results are expressed as means ± S.D. of three separate experiments.

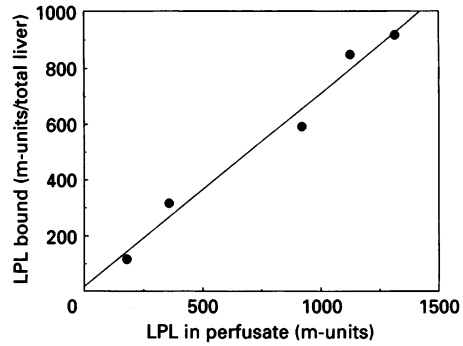

Figure 3 Unsaturable binding of lipoprotein lipase to perfused rat liver

Rat livers were perfused with media containing different amounts of lipoprotein lipase. Lipoprotein lipase activity was measured in the perfusate just before and after the recirculating perfusion. The measured differences in lipase activity are expressed as m-units of lipoprotein lipase (LPL) bound.

Table 1 Hepatic lipase binding in ACTH-treated rats

Livers of control and ACTH-rats were perfused with heparin- or 0.3 M NaCl-containing KRB. The perfusions were continued by recirculating perfusion with a hepatic-lipase-containing medium. Hepatic lipase activity was measured in the heparin and NaCl perfusates (HL released) and in the hepatic-lipase-containing media. The loss of lipase activity from the medium during the recirculating perfusion represents the bound lipase. The activities measured are expressed as m-units of lipase/g of liver. The Table shows means ± S.D. of 3–5 different experiments.

	Lipase activity (m-units/g of liver)	
	Controls	ACTH
HL released by heparin	763 ± 219	248 ± 61
HL binding after heparin pre-perfusion	90 ± 24	88 ± 12
HL released by 0.3 M NaCl	532 ± 154	152 ± 16
HL binding after NaCl pre-perfusion	541 ± 164	212 ± 60

Binding of hepatic lipase to heparin-pre-perfused liver

The obvious lack of binding of hepatic lipase to intact livers raised the question whether the binding capacity of the liver was completely utilized or whether the hepatic lipase preparations used were unable to bind to the liver in the experimental set-up. Therefore, livers were pre-perfused with a heparin-containing medium during 5 min to deplete the extracellular pool of hepatic lipase. More than 80 % of the total lipase activity is released from the liver in this way (Table 1). The heparin pre-perfusion of the livers was followed by a perfusion with a hepatic-lipase-con-

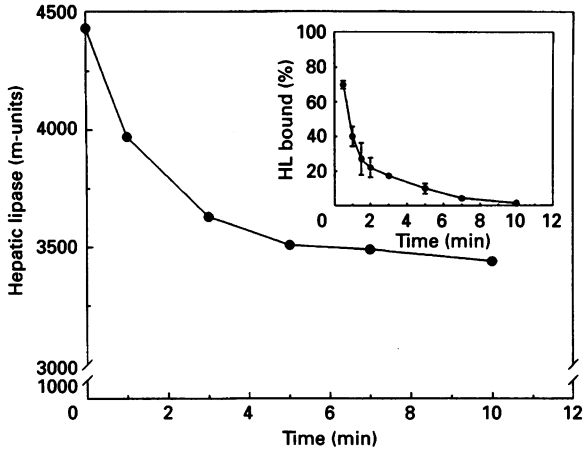

Figure 4 Binding of hepatic lipase to heparin-perfused livers

Rat livers that had been pre-perfused with heparin (5 units/ml) were perfused with media containing different amounts of hepatic lipase. Hepatic lipase was measured in the perfusate before and after the recirculating perfusion. The Figure represents a typical example of seven different experiments. In the insert, the time course of the binding of hepatic lipase (HL) is given. Hepatic lipase activity was measured in samples of the perfusate just before and after the passage through the liver. The portacaval differences (% of hepatic lipase activity bound) in lipase activities are expressed as % of the hepatic lipase in the medium, just before passage through the liver. Values in the insert are the means ± S.D. of 7 different experiments.

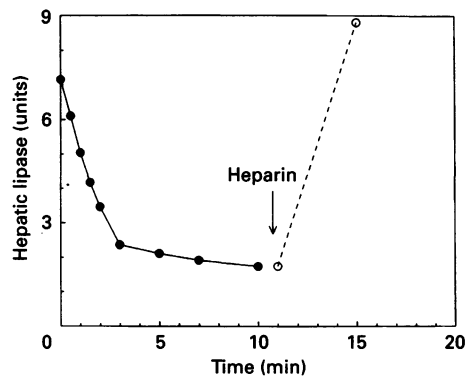

Figure 5 Binding of hepatic lipase to salt perfused livers

Rat livers that had been pre-perfused with 0.3 M NaCl were perfused in recirculating mode with media containing hepatic lipase. Hepatic lipase activity was measured in the recirculating perfusate (●). After the recirculating perfusion, livers were washed and perfused with heparin (5 units/ml) during 5 min. Hepatic lipase was also measured in the perfusate at the end of the recirculating perfusion and at the end of the heparin perfusion (○). The Figure represents a typical example of four different experiments.

taining medium as described above. Up to 1000 m-units of hepatic lipase activity were lost from the medium (Figure 4). The binding of the lipase was very rapid. During the first 30 s 80% of the lipase activity perfused through the liver was bound. After the first 1 min the percentage of the lipase bound during a single passage rapidly declined (Figure 4 insert). The amount of lipase activity bound did not increase even if more than 4000 m-units of hepatic lipase was perfused through the livers. The hepatic lipase activity that was bound to the liver during the perfusion could be completely recovered in the medium by heparin perfusion. The

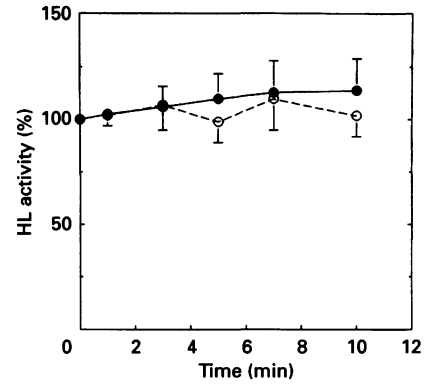

Figure 6 Binding of hepatic lipase (HL) to perfused livers: effect of ACTH treatment

Livers of control (●) and ACTH-treated (○) rats were perfused with media containing hepatic lipase as described in the legend to Figure 1. The Figure shows means ± S.D. of three different experiments.

maximal amount of hepatic lipase which bound to the liver was 10–20% of the activity originally present in the liver (Table 1: 90 ± 24 m-units/g of tissue). These results indicated that hepatic lipase from the medium can be bound to the liver during perfusion, but that the capacity of the liver to bind hepatic lipase is fully utilized in control rats. In addition, it appears that heparin lowers the binding capacity of the livers greatly, possibly by removing a hepatic-lipase-binding component from the liver.

NaCl perfusion

In view of these findings, we investigated the possibility of removing hepatic lipase from the liver without affecting the binding capacity. In a series of experiments using 0.15 M to 0.75 M NaCl in the perfusion medium, we found that 0.3 M NaCl in the medium released 60% of the liver hepatic lipase activity into the perfusate within 5 min (Table 1). Upon subsequent perfusion with heparin the remaining hepatic lipase activity (about 20%) was released from the liver.

Perfusion of 0.3 M NaCl-pre-perfused livers with a hepatic-lipase-containing medium led to a fall in hepatic lipase activity in the medium by 5000 m-units (Figure 5). This lipase activity could be recovered in the medium by heparin perfusion (Figure 5). Moreover, it can be seen that the amount of hepatic lipase activity released by 0.3 M NaCl perfusion equals the amount of hepatic lipase bound after subsequent perfusion of these salt-pre-perfused livers with hepatic-lipase-containing medium (532 ± 154 and 541 ± 164 respectively).

Hepatic lipase binding in control and ACTH-pre-treated rats

Previously we have shown that ACTH treatment leads to a diminished hepatic lipase activity in rat liver [12,30]. To study whether under these conditions binding capacity of the liver for hepatic lipase is also saturated, we performed perfusion experiments as described above with livers of ACTH-treated animals. During perfusion of ACTH-pre-treated livers with a hepatic-lipase-containing medium, no change in hepatic lipase activity occurred, indicating a complete utilization of the hepatic-lipase binding capacity of the livers (Figure 6). Subsequently, we studied binding of hepatic lipase to heparin-pre-perfused and to 0.3 M NaCl-pre-perfused livers in ACTH-treated animals. Hep-

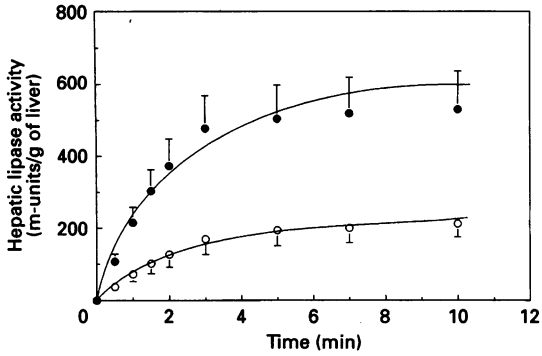

Figure 7 Binding of hepatic lipase to salt-pre-perfused livers: effect of ACTH treatment

Livers of control (○) and ACTH-treated (●) rats, pre-perfused with 0.3 M NaCl, were perfused in recirculating mode with hepatic-lipase-containing media. At different time intervals, hepatic lipase activities were measured in the media. The loss of lipase activity from the medium, divided by the liver weight, represents the amount of lipase activity bound per g of liver. The Figure shows mean ± S.D. of 3–5 different experiments.

arin perfusion released 248 ± 61 m-units/g of liver from the livers (Table 1). Perfusion of these livers with hepatic lipase led to a binding of 88 ± 12.4 m-units of hepatic lipase/g of liver, which is equal to the amount of hepatic lipase that can be bound to a heparin-pre-perfused control (not ACTH-pre-treated) liver. Perfusion of ACTH-pre-treated livers with 0.3 M NaCl released 152 ± 16 m-units of hepatic lipase/g of liver (Table 1). Perfusion of these salt-pre-perfused livers with a medium containing 5000–8000 m-units of hepatic lipase led to a fall in activity in the medium of about 2500 m-units (Table 1). Comparison of the binding of hepatic lipase to the livers after the 0.3 M NaCl perfusion between control and ACTH-rats revealed a 70 % lower maximal binding in the ACTH-rats than in the controls (Figure 7).

DISCUSSION

In previous studies, using isolated non-parenchymal liver cells, a decrease in the amount of specific binding sites was observed on non-parenchymal liver cells isolated from ACTH-treated rats [12]. A decrease in the amount of binding sites for hepatic lipase is hardly of physiological importance if only a few binding sites are occupied. A decrease in the binding capacity will be of much more importance for the expression of hepatic lipase activity if all binding sites are occupied. In this paper we show that in normal rat liver all binding sites for hepatic lipase are occupied *in situ*, as at both 4 °C and 37 °C no additional binding of hepatic lipase was observed if rat livers were perfused with hepatic-lipase-containing media. Also, in livers of ACTH-rats no binding of hepatic lipase was found during perfusion with lipase-containing medium. In these livers total extracellularly located lipase activity is greatly lowered [12,30]. We have previously shown by immunotitration that the specific activity of hepatic lipase in ACTH-rats is not altered [12]. Therefore it can be concluded that also in livers of ACTH-rats all binding sites are occupied, indicating a lowered number of binding sites. The complete occupancy of the hepatic-lipase binding sites indicates that a decrease in the binding capacity leads to a decreased activity of the enzyme. In contrast with hepatic lipase, lipoprotein lipase did bind to normal rat liver. More than 80 % of lipoprotein lipase was bound under the perfusion conditions used. The fact that lipoprotein lipase, but not hepatic lipase, binds to perfused liver shows for the first time that the binding site for hepatic lipase is different from the hepatic binding site for lipoprotein lipase, suggested previously on the basis of the different disappearance rates of the two enzymes after injections of several size-fractionated heparins [38]. If hepatic lipase, as generally assumed, binds to heparan sulphate proteoglycans, the structural characteristics of the proteoglycan(s) involved must be highly specific. In contrast with lipoprotein lipase, which is bound to most tissues and cells, hepatic lipase is only bound to the liver, adrenal cortex and ovary [39,40]. Liver contains about 400 μg of heparan sulphates [41]. Three forms of heparan sulphate proteoglycans have been isolated from rat liver, a peripheral proteoglycan, a membrane-intercalated proteoglycan and a proteoglycan associated with lysosomes [42,43]. Recently a novel heparan sulphate in the extracellular matrix and the basement membranes of the liver has been found [44]. The best characterized heparan sulphate proteoglycan localized on the sinusoidal rat liver plasma membrane has a molecular mass of 75 kDa [43,44]. Normal rat liver contains about 7500 m-units of hepatic lipase activity. Assuming a specific activity of hepatic lipase of 800 m-units/μg of protein and a molecular mass of 57 kDa [33,37], this amount of hepatic lipase corresponds to 0.165 nmol of hepatic lipase. If hepatic lipase is bound as a monomer in a 1 : 1 ratio to its binding site, 0.165 nmol of receptor molecules should be present in a whole rat liver. If this proteoglycan would function as the specific receptor for hepatic lipase in the liver, 0.165 nmol of receptor molecules correspond to 8 μg of heparan sulphate/total liver, indicating that only a small part of the heparan sulphates has the structural characteristics necessary for the binding of hepatic lipase. The nature of the hepatic-lipase binding sites is unknown. From the experiments presented it is tempting to suggest that hepatic lipase is bound in two ways to the liver. Part of the lipase (about 10 % in normal liver) seems to be bound to a site which remains intact after perfusion with heparin, representing a heparin-resistant or heparin-insensitive binding site. The other hepatic-lipase binding site is removed or inactivated by heparin, representing a heparin-sensitive or heparin-releasable binding site. Possibly, this binding site consists of a protein releasable from the liver by heparin, although inactivation of a binding site by heparin cannot yet be excluded. Since the heparin-resistant binding of hepatic lipase was similar in control and ACTH-rats, this type of binding site seems not to be regulated, at least under the conditions studied. It contributes from about 10 % in controls to about 30 % in ACTH-rats to the binding of the extracellularly located hepatic lipase pool. The heparin-releasable or heparin-sensitive binding site is greatly diminished in the ACTH-rats. The lowering of this binding completely accounts for the lowered hepatic lipase activity in the ACTH-rats. This suggests that via this binding the expression of hepatic lipase may be regulated, although regulation at the site of synthesis (secretion) of the lipase also occurs [12]. A heparin-releasable protein (HRP) binding lipoprotein lipase has been detected in bovine aortic endothelial cells [20]. Whether the presumed hepatic-lipase binding protein is similar to or even identical with this lipoprotein lipase binding protein is not clear. However, lipoprotein lipase is not bound to this protein in the liver, since the binding of lipoprotein lipase was not affected by prior heparin preperfusion. The presence of at least two different binding mechanisms of hepatic lipase to the liver raises questions about the functionality of the differentially bound enzymes in relation to the localization of the different binding sites. In addition, the finding that the heparin-sensitive binding is lowered by ACTH treatment opens the possibility that hepatic lipase expression in liver can be regulated via the binding capacity for the enzyme.

Studies are in progress in our laboratory to identify the specific binding sites for hepatic lipase.

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