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The Release of Hepatic Triglyceride Lipase from Rat Monolayered Hepatocytes in Primary Culture

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Abstract

The release of hepatic triglyceride lipase from cultured rat hepatocytes and its hormonal regulation were studied. The activity of lipase released into the medium in the presence of heparin was increasing during 24 hours on the 2nd day of culture, while this was 10% in the absence of heparin as compared with the lipase activity in the presense of heparin. When hepatocytes were cultured with anti-hepatic triglyceride lipase IgG, the lipase activity was suppressed by 92%. The results suggest that the enzyme released into culture medium is identical to hepatic triglyceride lipase which can be released only in the presence of heparin, the model of release being similar to that of lipoprotein lipase from adipocytes. The addition of monensin to the medium resulted in the inhibition of lipase secretion by 61%. Insulin enhanced lipase activity only 20%, whereas dexamethasone suppressed the activity by 44%. These data indicated that hepatic triglyceride lipase is secreted and released from hepatocytes in the presence of heparin and its secretion is regulated by hormones.

Introduction

Hepatic triglyceride lipase is the enzyme synthesized by hepatocytes[1-3] and then proceeded to the vascular endothelium in the liver[4]. The extracellular localization of the enzyme suggests an involvement of this enzyme in lipoprotein metabolism. We and others have suggested that this enzyme mediates the catabolism of remnant lipoproteins and high density lipoproteins(HDL)[5-7]. Both lipoproteins play important roles in atherogenesis : remnant lipoprotein accelerates atherosclerosis and HDL prevents it. However, the precise mechanism of hepatic triglyceride lipase secretion from liver and its regulation is not well understood. For studying the mechanism of hepatic triglyceride lipase secretion from hepatocytes, some investigators tried a short-term experiment using cell suspension[8,9]. Later, Leitersdorf et al.[10] studied the *regulation of synthesis and secretion of hepatic* triglyceride lipase using monolayered rat hepatocytes, and suggested that hepatic triglyceride lipase is heparin-releasable enzyme like lipoprotein lipase in adipocytes. There have been different results on a regulation of hepatic triglyceride lipase activity by hormones[1,11-13]. In the present study, we have examined the effects of heparin, hormones and some agents inhibiting protein secretion of hepatic triglyceride lipase from hepatocytes in primary culture.

Materials and Methods

Materials

Male Wister rats(150~180 g) fed ad libitum were used. Collagenase(type I), insulin, dexamethasone, colchicine, monensin and triolein was obtained from Sigma Chemical Co., St. Louis, MO and heparin was from Novo Indust., Denmark. Ham's F12 medium, fetal bovine serum, penicillin, streptomycin and fungizone were obtained from Gibco Laboratories, Grand Island, NY.

Primary culture of hepatocytes

Primary culture of hepatocytes was performed according to the method of Berry and Friend[14]. Rat liver was perfused with a solution containing collagenase in Hanks' buffered saline(150 mg/150 ml) for 10~15 min at 37℃ in a 95% O_2 and 5% CO_2. After perfusion, hepatocytes were suspended in Ham's F12 medium containing 10% fetal bovine serum, 10^{-9} M insulin, penicillin-streptomycin-fungizone(10,000 unit, 10 mg and 25 μg/100ml medium, respectively). The suspension was centrifuged at 50×g at 4℃ for 1 min and the cells centrifuged were resuspended in the above medium. The washing was repeated 4 times, and then the viability of cells in final suspension was checked using trypan blue. We used the cells for the study when more than 85% of the isolated cells excluded trypan blue. The cells were plated in 60 mm collagen-coated plastic dish at 1×10^6 cells/ml and placed in incubator at 37℃ in a 95% air, 5% CO_2. After 8 hours(the cells are attached to the dish 4 hours after plating), the medium was replaced by hormone-free medium, and 16 hours later heparin and the hormones to be examined were added to the medium. Then the cells were cultured for another 24 hours. After having finished, the culture medium was used for the measurement of triglyceride lipase activity, and the cells were sonicated to determine protein concentration[15].

Triglyceride lipase activity

Triglyceride lipase activity was measured with minor modification of the previously described method[16, 17]. The substrate was a mixture of 2 μCi of glycerol tri [1-^{14}C]oleate(Amersham/Seale Corp. Ill.), 0.133 g of unlabeled triolein, 0.3% bovine serum albumin and 0.075% Triton X-100, diluted with 0.02 M Tris-HCl, pH 8.6, to a final volume of 12 ml. 0.4 ml of the above sonicated substrate and 0.6ml of cultured medium was incubated for 30 min at 37℃. Free fatty acids(FFA) liberated from glycerol tri [1-^{14}C]oleate during incubation were extracted and the enzyme activity was expressed as nmoles FFA/min/mg cell protein.

Anti-hepatic triglyceride lipase serum was produced against hepatic triglyceride lipase purified about 1,000-fold from rat postheparin plasma using heparin-Sepharose 4B affinity chromatography as mentioned previously[7].

IgG fraction of this antiserum was prepared using protein A-Sepharose CL 4B affinity chromatography. One ml of IgG solution contains an amount of IgG in 2 ml of antiserum. Specificity of the antibody to hepatic triglyceride lipase has been described previously[18].

Results and Discussion

In the present study, we used rat monolayered hepatocytes in primary culture in order to study the regulation of hepatic triglyceride lipase secretion. There are two advantages of using culture system : the collagenase-digested cell membrane can be restored during cell culture and cell functions can be maintained at steady state for a study period.

In our culture system, the amounts of lipase released into the medium were increasing during 24 hours in the presence of heparin(Fig. 1). The

steep increase in the lipase activity for the first 2-4 hours may reflect mainly the acute release of membrane-bound lipase. We could not detect this steep increase on the 1st day of culture, indicating that the function of cell membrane to secrete enzyme was not recovered after digestion of cells with collagenase. The activity of triglyceride lipase released by heparin into the medium during 24 hours on the 2nd day of culture was 3.8 nmoles FFA/min/㎎ cell protein. Sundaram et al.[1] and Jansen et al.[2] have reported that even without heparin a large amount of lipase could be released from isolated hepatocytes in a short-term experiment using cell suspension. However our results indicate that the release of triglyceride lipase in the absence of heparin was markedly decreased (〈10%) compared with that in the presence of heparin. The addition of heparin to this heparin-free culture medium, of course, produced a large and

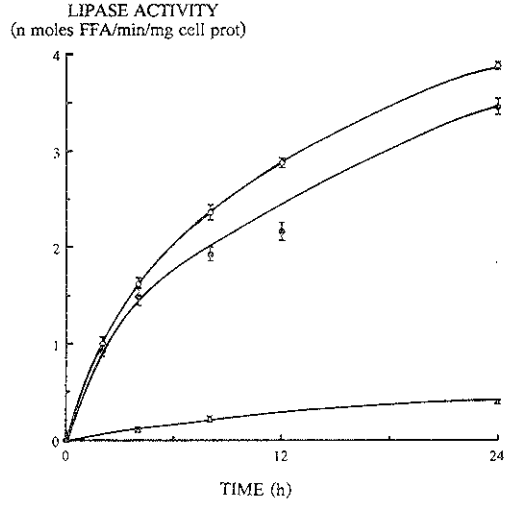

Fig. 1. The time-related change in hepatic triglyceride lipase activity. Monolayered hepatocytes were used for the experiment on the 2nd day of primary culture. The experiments were done in the presence of heparin (10 U/㎖ medium) with(－◦－) or without(－•－) addition of insulin (10^{-7} M). These were also done in the absence of heparin with insulin(－△－). The results were expressed as mean± SD(n=5).

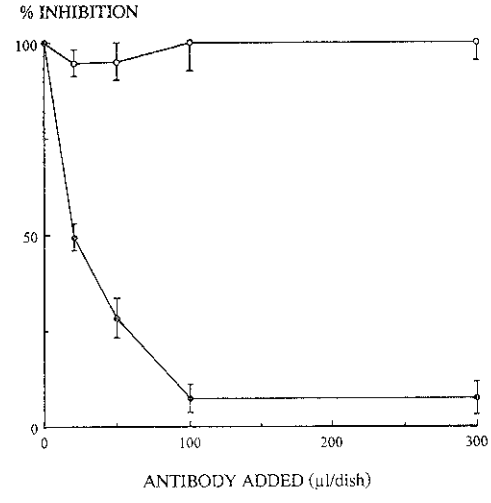

Fig. 2. Effect of anti-hepatic triglyceride lipase IgG on the activity of triglyceride lipase released from hepatocytes in primary culture. The cells were incubated with IgG on the 2nd day of culture(－◦－ : normal rabbit IgG, －•－ : anti-hepatic triglyceride lipase IgG). The results were expressed as mean± SD(n=5).

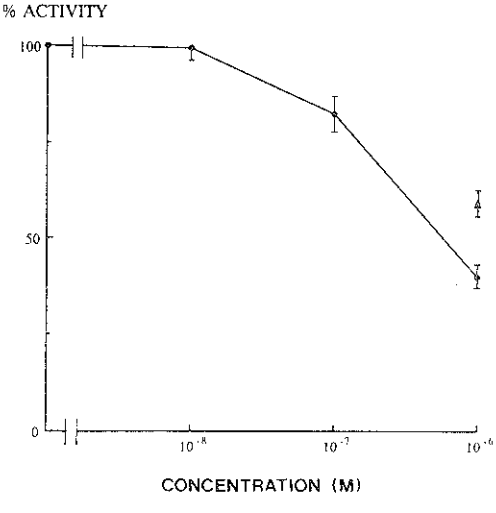

Fig. 3. Effect of monensin(－•－) and dexamethasone(－▲－) on the activity of hepatic triglyceride lipase released from monolayered hepatocytes. Experiments were done in the presence of heparin(10 U/㎖ medium) with addition of insulin(10^{-7} M). The results were expressed as mean± SD (n=5).

sharp increase in the release of enzyme. These results suggest that triglyceride lipase synthesized in the liver is transported to hepatocyte membrane where the enzyme is bound, and then the enzyme is released by heparin. The proposed mechanism is very similar to that of lipoprotein lipase from fat cells[19] and heart cells[20].

Our data support the finding by Leitersdorf et al.[10] in which heparin enhances the release of hepatic triglyceride lipase from the cell surface of hepatocytes. Based on the observation that the release of hepatic triglyceride lipase is very limited in the absence of heparin, the following studies were carried out with addition of heparin(10 U/mℓ medium).

When monolayered hepatocytes were cultured with anti-hepatic triglyceride lipase IgG, the activity of triglyceride lipase in culture medium was suppressed by 92%, while the addition of normal rabbit IgG to the medium did not show any effects on the lipase activity(Fig. 2). This indicates that the triglyceride lipase released from cultured hepatocytes is immunologically identical with hepatic triglyceride lipase found in postheparin plasma.

Using monolayered hepatocytes, the effects of two agents inhibiting protein secretion on the lipase secretion were studied. One in colchicine(10^{-4} M) which blocks polymerization of microtubules, and the other is monension(10^{-4} M) which is a carboxylic ionophore and disrupts the movement of secretory proteins from the Golgi apparatus to the plasma membrance[21]. Both colchicine (data not shown) and monensin suppressed the release of hepatic triglyceride lipase by $20\pm3\%$ (mean$\pm$SD ; n=5) and $61\pm3\%$(n=5), respectively($P<0.001$ for both)(Fig. 3). Our data obtained from the colchicine study indicating that the secretion of hepatic triglyceride lipase partially involves microtubular transport mechanism is consistent with the in vivo observation by Chajek et al.[22]on the hepatic triglyceride lipase in rat postheparin plasma. The marked inhibition of the lipase secretion by monensin suggests that process of hepatic triglyceride lipase secretion depends on an ionic gradient across membrane.

The addition of insulin at concentrations of 10^{-9} and 10^{-7} M to the medium increased the heparin-releasable hepatic triglyceride lipase by 17 ± 5 and $20\pm6\%$, respectively($P<0.01$ for both ; n=5) (data not shown). Different data have been reported previousiy on the activity of hepatic triglyceride lipase in rats with experimental diabetes melliuts : this was unchanged[11] or decrease[12,13]. Although insulin increased hepatic triglyceride lipase activity significantly in the present study, this was minimal and we can not conclude from our data that hepatic triglyceride lipase is an insulin-dependent enzyme.

Dexamethasone(10^{-6} M) suppressed markedly hepatic triglyceride lipase activity($44\pm5\%$, $P<0.001$; n=5). The finding is consistent with the data obtained by Sundaram et al.[1] from short-term incubation studies using hepatocyte suspension. In the present study, we demonstrated the effects of insulin and dexamethasone on hepatic triglyceride lipase activity. However, factors regulating this enzyme activity have not yet been assessed fully. Cultured hepatocytes would permit further studies to understand its metabolic regulation.

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일차배양 쥐간세포로부터 간트리글리세리드 lipase의 유리

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요 약

쥐간세포 배양시 간트리글리세리드 lipase의 유리 및 호르몬 조절에 관하여 연구하였다. 배양 2일째 헤파린 첨가구 배양액에 유리된 lipase 활성은 24시간 동안 계속 증가하였다. 반면에 헤파린 무첨가의 lipase활성은 헤파린 첨가구에 비하여 10%에 지나지 않았다. 간세포를 anti-hepatic triglyceride lipase IgG와 배양시 lipase 활성이 92%까지 저해되었다. Monensin 첨가시 lipase활성 저해는 61%였다. 인슐린은 lipase활성을 20% 상승시켰으며 dexamethasone은 44% 저해시켰다. 이상의 결과로 미루어 보아 간트리글리세리드 lipase는 헤파린 존재하에 분비 및 유리되며 그 분비는 호르몬에 의해 조절됨을 시사한다.