Stimulatory Release of Hepatic Lipase Activity from Liver by Epidermal Growth Factor

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We have found that release of hepatic lipase activity is stimulated from liver slices by epidermal growth factor (EGF) into the medium in a time-dependent manner. This novel effect of EGF was markedly decreased by various tyrosine kinase inhibitors, such as α -cyano-3-ethoxy-4-hydroxy-5-phenyl-thiomethyl cinnamamide, amiloride and biochanin A. The stimulation by EGF was also suppressed, however, by dibutyl cyclic adenosine monophosphate, and 3-isobutyl-1-methylxanthine, a cyclic nucleotide dependent phosphodiesterase inhibitor.

These findings show that the stimulatory release of the enzyme activity by EGF is associated with the activation of tyrosine kinase activity and with the intracellular cyclic adenosine monophosphate level.

Keywords hepatic lipase; epidermal growth factor; tyrosine kinase; cyclic adenosine monophosphate

Introduction

Hepatic lipase (HTGL; hepatic triacylglycerol lipase, EC 3.1.1.3) hydrolyzes chylomicron remnant, very low- and intermediate-density lipoprotein triacylglycerols, and low density lipoprotein and high density lipoprotein phospholipids.^{1–6} It has been reported that the release of HTGL activity is accelerated by heparin and vanadate, and reduced by adrenalin and vasopressin.^{1,7,8} However, there are only a few reports on the relationship between the enzyme release and growth factors.

Epidermal growth factor (EGF) stimulates the physiological responses including epidermal proliferation, amino acid uptake, hexose transport, and phosphatidylinositol turnover.^{9–11} It has also been shown that EGF activates the phosphorylation of proteins on tyrosine residues, that EGF receptor itself is probably the tyrosine-specific protein kinase (TK), and that its kinase activity is increased as a result of EGF binding to the receptor.^{11–14} We recently suggested the possibility that HTGL activity is released by the activation of TK activity.⁷ In this study, we show that the release of HTGL activity from the isolated rat liver is stimulated by EGF.

Materials and Methods

Materials [γ -³²P]Adenosine triphosphate (ATP, 111 TBq/mmol) was obtained from New England Nuclear (MA, U.S.A.). EGF (mouse submaxillary gland), dibutyl cyclic adenosine monophosphate (Dib. cAMP) and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Wako Chemical Industries (Osaka, Japan). Amiloride, biochanin A and poly (glutamate:tyrosine; 4:1) were obtained from Sigma (MO, U.S.A.). α -Cyano-3-ethoxy-4-hydroxy-5-phenyl-thiomethyl cinnamamide (ST 638)^{15–17} was provided by Dr. Tadayoshi Shiraishi (Kanegafuchi Chemical Industry Co., Osaka). Intralipos, an emulsion composed of 1.2% yolk lecithin, 2.5% glycerol, and 10% soybean oil, was from Green Cross Co. (Osaka). All other chemicals used were of analytical grade.

Animals Male Wistar rats weighing 250–300 g were kept on a commercial laboratory chow *ad libitum* for one week and starved for 24 h before the experiments.

Determination of HTGL and TK Activity As previously described,^{7,18} the liver was quickly removed from rats killed under anaesthesia, then the tissue was sliced with a razor into small pieces (100–150 mg). Three slices (400 mg total) were incubated in 2 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) containing 5 mM glucose and 2% bovine serum albumin under 95% O₂–5% CO₂ in siliconized vials at 28 °C for 120 min. After incubation, the liver and the medium were separated by centrifugation and the obtained supernatant served as the preparation of the released HTGL activity.⁷ The enzyme activity was determined by the method

using Intralipos as substrate.¹¹ The free fatty acids (FFA) produced were determined by Duncombe's method.¹⁹ The HTGL activity was expressed as μ mol FFA produced/h/g liver. The liver was homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 0.25 M sucrose and centrifuged as described previously.⁷ The resultant supernatant served for the measurement of TK activity by the method of Braun *et al.*²⁰ using poly (glutamate:tyrosine; 4:1) and [γ -³²P]ATP. Results are means $\pm$ S.E. of four or five observations for two separate experiments.

Results and Discussion

Stimulatory Release of HTGL Activity from Liver by EGF The appearance of HTGL activity in the medium was observed in the presence of EGF (25 nM) over a 150-min incubation period (Fig. 1). The EGF-stimulated release of HTGL activity reached its maximum in a 120-min period, then maintained a steady-state level up to 150 min.

Effects of Various TK Inhibitors To confirm whether TK activity is involved in the stimulatory release of HTGL activity by EGF, the liver was incubated with EGF in the presence of various tyrosine kinase inhibitors such as ST 638 (4-hydroxycinnamamide derivative),^{15–17} amiloride (pyrazine derivative)²¹ and biochanin A (isoflavone derivative)²² (Table I). The stimulatory release of HTGL activity and the increase of TK activity by EGF was markedly suppressed by each inhibitor. These findings

Fig. 1. Time Course of Release of HTGL Activity from the Liver by EGF

The liver was incubated for 0–150 min with (●) or without (○) EGF (25 nM). HTGL activity released into the medium was determined as described in Materials and Methods.

TABLE I. Effects of Various TK Activity Inhibitors on Release of HTGL Activity and Increase of Tyrosine Kinase Activity by EGF

EGF (25 nM)	Activity (%)	
	HTGL	TK
None	100	100
ST638 (1 mM)	7 ± 0.5	18 ± 2.1
Amiloride (3 mM)	15 ± 0.9	23 ± 2.6
Biochanin A (150 µg/ml)	10 ± 0.6	13 ± 0.5

The liver was incubated for 120 min with or without EGF (25 nM) in the presence of ST 638, amiloride or biochanin A at the indicated concentration. No significant change in basal activity of HTGL and TK was found with any inhibitor alone. The net release of HTGL activity and increase of TK activity by EGF (25 nM) were 0.20 ± 0.01 (µmol FFA/h/g liver) and 3.0 ± 0.14 (fmol $\times 10^2$ /min/mg protein), respectively. Percentage value was calculated from each net activity.

TABLE II. Effects of Dib. cAMP and IBMX on Stimulatory Release of HTGL Activity by EGF

Chemical (mM)	HTGL activity (µmol FFA/h/g liver)	
	-EGF	+EGF
None	0.44 ± 0.03	0.61 ± 0.03 ^{a)}
Dib cAMP 0.2	0.44 ± 0.02	0.51 ± 0.04 ^{b)}
0.5	0.45 ± 0.03	0.49 ± 0.03
IBMX 0.1	0.44 ± 0.03	0.55 ± 0.05 ^{b)}
0.3	0.43 ± 0.04	0.48 ± 0.03

The liver was incubated for 120 min with or without EGF (25 nM) in the presence of Dib. cAMP and IBMX at the indicated concentration. Significantly different from no EGF at $p < 0.01$ a) and $p < 0.05$ b).

suggest that the stimulatory release of HTGL activity by EGF is associated with an activation of the kinase activity via a ST 638-, amiloride-, and biochanin A-sensitive process.

Involvement in Intracellular Cyclic Nucleotide To study whether changes in the intracellular cAMP level affect the stimulatory release of HTGL activity by EGF, the liver was incubated with EGF in the presence of Dib. cAMP or IBMX (Table II). IBMX is a well-known potent inhibitor of cyclic nucleotide dependent phosphodiesterase²³⁾ and we recognized that IBMX increased cAMP level from 0.22 ± 0.02 to 0.48 ± 0.05 nmol/g liver under our experimental conditions using the radioimmunoassay (cAMP-[¹²⁵I] assay system; Amersham Japan, Tokyo). An inhibition of the EGF action was observed with Dib. cAMP and IBMX. These results show that the stimulatory release by EGF is inhibited by a rise in intracellular cAMP level. It has been reported that Dib. cAMP plus theophylline inhibits the stimulation of DNA synthesis by EGF in human fibroblasts.²⁴⁾ The secretion of lipoprotein lipase activity from cultured avian adipocytes also was inhibited by Dib. cAMP.²⁵⁾ Therefore, the inhibition of the stimulatory release of HTGL activity by the intracellular

increase in cAMP level may be due to changes in the rate of synthesis or degradation of the HTGL molecule and/or a modification of the fully active enzyme to a less active one. The EGF concentration in mouse plasma is reported to be ca. 0.2 nM, and to be able to increase to 30 nM after administration of an α -adrenergic agent.⁹⁾ It is also considered from physiological aspects that the release of HTGL activity is stimulated by EGF at the concentration used in this report. Further details, however, are being gathered. The first step in the EGF signal transduction pathway is reported to be the activation of the tyrosine kinase activity following its receptor binding.¹¹⁾

In conclusion, we have shown that the stimulatory release of HTGL activity from liver is stimulated by EGF, and that it may be caused by the activation of TK activity and a sensitization process to the intracellular cAMP level.

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