

Endothelin-1 Stimulates Secretion of Lipoprotein Lipase from Ehrlich Ascites Tumor Cells

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Since the role of endothelin (ET)-1 in lipoprotein metabolism in tumor cells is unclear, we investigated the effect of ET-1 on the secretion of lipoprotein lipase (LPL) from mouse Ehrlich ascites tumor cells. ET-1 increased the secretion of LPL from these cells in a time-dependent manner. Two antagonists of ET-receptor type A (ET-A), namely, BQ123 and FR139317, inhibited the stimulatory effect of ET-1 on the secretion of LPL. However, an antagonist of ET-receptor type B (ET-B), BQ788, did not have any effect. Neomycin, a phospholipase C (PLC) inhibitor, and H-7, a protein kinase C (PKC) inhibitor, also suppressed the ET-1-stimulated secretion of LPL. ET-1 also increased PKC activity in tumor cells in a dose-dependent manner. These results imply that ET-1 stimulates secretion of LPL from tumor cells by stimulating the PLC-PKC signaling pathway through the ET-A receptor rather than the ET-B receptor.

Key words— lipoprotein lipase, endothelin-1, endothelin receptor, Ehrlich ascites tumor

INTRODUCTION

The functions of lipids in supplying energy and structural components of cell membranes are important in tumor growth and proliferation. However, the role of lipid metabolism in cancer cells is not fully understood. In lipid metabolism, lipoprotein lipase (LPL; EC 3.1.1.34) catalyzes the hydrolysis of the triacylglycerol component of very low density

lipoproteins in plasma, thereby regulating the cellular absorption of free fatty acids and higher density lipoproteins.¹⁾ LPL adheres to cell surfaces and to the luminal endothelium of blood vessels.²⁾ LPL is also found in Ehrlich ascites tumor cells, where its activity is correlated with the uptake of lipids.³⁾

Endothelin (ET)-1 is a 21-amino acid vasoconstricting peptide, which plays an important role in vascular homeostasis.^{4,5)} There are two types of receptors for ET-1, namely, ET-A and ET-B.^{6–8)} The ET-A receptor is found in smooth muscle tissue of blood vessels and the ET-B receptor is found in vascular endothelial cells.^{5,9)} When ET-1 binds to the ET-A receptor, vasoconstriction and increased retention of sodium lead to an increase in blood pressure.¹⁰⁾ In contrast, when ET-1 binds to the ET-B receptor, increased natriuresis, diuresis, and release of nitric oxide result in lower blood pressure.¹¹⁾

Few studies have investigated the effects of ET-1 on lipoprotein metabolism, particularly the secretion of LPL from tumors. In this study, we show that ET-1 stimulates the secretion of LPL from mouse Ehrlich ascites tumor cells and that this effect is associated with an increase in protein kinase C (PKC) activity through the ET-A receptor.

MATERIALS AND METHODS

Materials— The PKC enzyme immunoassay system (MESACUP) was obtained from Medical & Biological Laboratories (Nagoya, Japan). ET-1 was purchased from Peptide Institute, Inc. (Osaka, Japan). BQ123 and BQ788 were obtained from Funakoshi Co. Ltd. (Tokyo, Japan). FR139317 was bought from Fujisawa Pharmaceutical Co. Ltd. (Tokyo, Japan). Neomycin was purchased from Wako Pure Chemical Industries (Osaka, Japan). H-

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7 was obtained from Seikagaku Industries (Tokyo, Japan). Intralipos, an emulsion of 1.2% yolk lecithin, 2.5% glycerol, and 10% soybean oil, was purchased from Otsuka Pharmaceutical Co. Ltd. (Tokyo, Japan). All other chemicals used were of analytical grade.

Preparation and Incubation of Ehrlich Ascites Tumor Cells— Male ddY mice (25–30 g) were fed commercial laboratory chow *ad libitum*. Ehrlich ascites tumor cells were transplanted intraperitoneally into these mice (1×10^8 cells each).¹² These cells were harvested 10–14 days after transplantation, and washed twice with Krebs-Ringer bicarbonate buffer (pH 7.4) before using them in experiments. The cells were incubated at 37°C in 2 ml Krebs-Ringer bicarbonate buffer containing 5 mM glucose and 2% bovine serum albumin in siliconized vials in a 95% O₂/5% CO₂ atmosphere. After incubation, the cells were centrifuged and the supernatant was collected for measuring LPL activity. The cell pellet was homogenized in Krebs-Ringer bicarbonate buffer (pH 7.4) and centrifuged at 15000 *g* for 10 min at 4°C. The supernatant was collected for determining PKC activity.

Determination of LPL and PKC Activity— LPL activity was measured by a colorimetric method using Intralipos as a substrate.^{12, 13} LPL activity was expressed as nmol of free fatty acids (FFA) produced/h per 10⁶ cells. PKC activity was determined using the MESACUP Protein Kinase Assay Kit (Medical & Biological Laboratories). Briefly, the kit is based on enzyme linked immunosorbent assay that utilizes the peptide synthesized as a substrate for PKC and a monoclonal antibody recognizing phosphorylated form of the peptide. PKC present in samples catalyzes phosphorylation of the synthetic peptide coated on microwell. The biotinylated monoclonal antibody is bound to the phosphorylated peptide, and is subsequently detected with streptavidin conjugated to peroxidase. Peroxidase substrate solution (H₂O₂ and *o*-phenylenediamine) is then added, and the intensity of the color is measured photometrically at 492 nm as PKC activity.

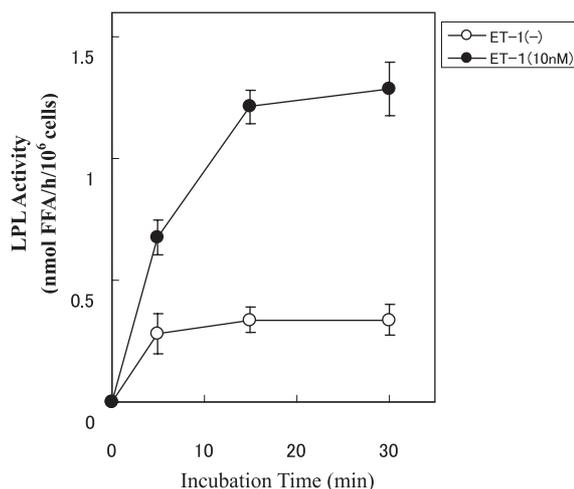
Data Analysis— Experimental results are expressed as the mean \pm S.D. of four or five measurements using different tumor cell samples.

RESULTS

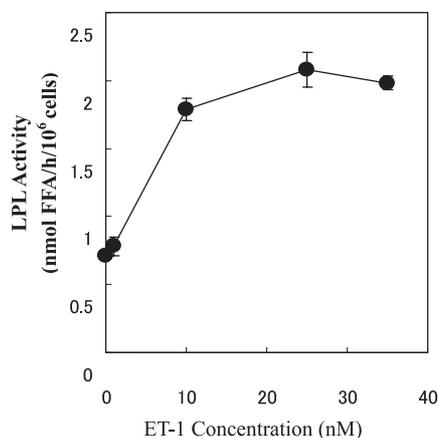
LPL was secreted from Ehrlich ascites tumor

cells into the growth medium after incubating with 10 nM ET-1 for 30 min. ET-1 stimulated LPL secretion in a time-dependent manner (Fig. 1a). When Ehrlich ascites tumor cells were incubated with increasing concentrations of ET-1, LPL secretion increased in a dose-dependent manner (Fig. 1b). This increase was also associated with a decrease in intracellular LPL activity (data not shown).

To determine whether ET-1 activated ET-A or ET-B receptors, the tumor cells were incubated with ET-1 in the presence of either ET-A receptor antagonists BQ123 or FR139317,^{14–16} or the ET-B receptor antagonist BQ788.^{17–19} ET-1-stimulated secretion of LPL decreased when treated with 1 μ M BQ123 or FR139317. However, BQ788 did not



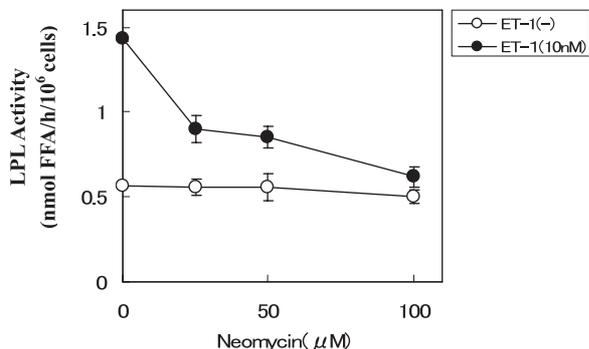
(a) Ehrlich ascites tumor cells were incubated with (●) or without (○) 10 nM ET-1 for 30 min.



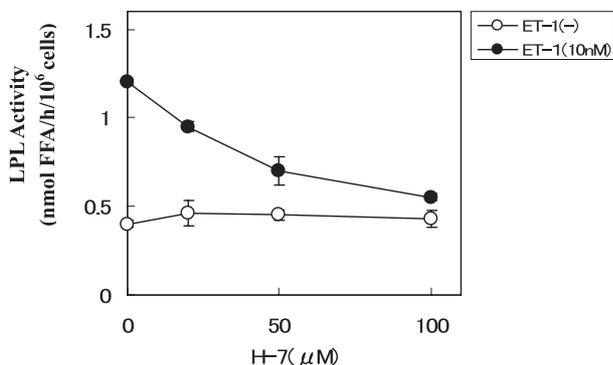
(b) The tumor cells were incubated for 30 min with various concentrations of ET-1.

Fig. 1. Stimulated Secretion of LPL from Ehrlich Ascites Tumor Cells by ET-1

The activity of LPL secreted into the growth medium was measured as described in MATERIALS AND METHODS.



(a) Ehrlich ascites tumor cells were incubated for 30 min with (●) or without (○) 10 nM ET-1 in the presence of neomycin.



(b) The tumor cells were incubated for 30 min with (●) or without (○) 10 nM ET-1 in the presence of H-7.

Fig. 2. Effects of PLC and PKC Inhibitors on the ET-1-Stimulated LPL Secretion

The activity of LPL secreted into the growth medium was measured as described in MATERIALS AND METHODS.

Table 1. Effects of ET Receptor Antagonists on LPL Activity

Antagonist	LPL Activity (nmol FFA/h per 10 ⁶ cells)	
	(-)ET-1	(+)ET-1 (10 nM)
None	0.53 ± 0.06	1.17 ± 0.11
BQ123 (1 μM)	0.52 ± 0.04	0.59 ± 0.05
FR139317 (1 μM)	0.52 ± 0.04	0.56 ± 0.04
BQ788 (1 μM)	0.53 ± 0.05	1.14 ± 0.09

Ehrlich ascites tumor cells were incubated for 30 min with or without 10 nM ET-1 in the presence of various antagonists, and the activity of LPL in the growth medium was then measured as described in MATERIALS AND METHODS.

have any effect on the stimulation of ET-1 (Table 1).

The effect of phospholipase C (PLC) and PKC inhibitors on ET-1-stimulated LPL secretion was also investigated. Specifically, neomycin²⁰ and H-7,^{21,22} inhibitors of PLC and PKC respectively, suppressed ET-1-stimulated LPL secretion (Fig. 2a and 2b).

Finally, to determine whether ET-1 affected PKC activity, the tumor cells were incubated for

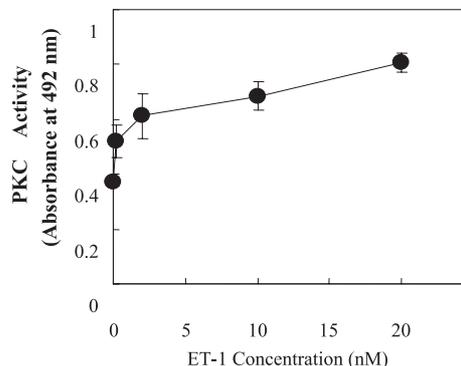


Fig. 3. Effect of ET-1 on PKC Activity in Ehrlich Ascites Tumor Cells

Ehrlich ascites tumor cells were incubated for 30 min with various concentrations (0–20 nM) of ET-1. PKC activity was measured as described in MATERIALS AND METHODS.

30 min with ET-1, and PKC activity was then measured. PKC activity increased in a dose-dependent manner up to 20 nM ET-1 (Fig. 3).

DISCUSSION

LPL is a key enzyme in lipid metabolism and plays an important role in tumor growth. It is also a specific marker of adipogenic differentiation. ET-1 may inhibit the differentiation of brown adipocytes by modulating LPL secretion and activity.²³ Specifically, ET-1 suppresses the release of LPL by heparin and reduces the activity of LPL remaining in the cell extracts of brown adipocytes. Moreover, pretreatment of brown adipocytes with ET-A receptor antagonists blocks the latter effect.

In this study, ET-1 stimulated LPL secretion from Ehrlich ascites tumor cells (Fig. 1a and 1b). Two ET-A receptor antagonists suppressed the stimulatory effects of ET-1, but an ET-B receptor antagonist had no effect (Table 1). Furthermore, PLC and PKC inhibitors suppressed ET-1-stimulated LPL secretion (Fig. 2a and 2b). These results suggest that ET-1 increases PLC activity, which in turn increases PKC activity in tumor cells (Fig. 3). This is consistent with the observation that ET-1 activates PKC α in cultured cat iris sphincter smooth muscle cells.²⁴

ET-1 has important functions in tumor growth and progression. For example, ET-1 plays an important role in the progression of breast cancer and is overexpressed in breast carcinomas, along with the ET-A and ET-B receptors.²⁵ Moreover, YM598, an inhibitor of the ET-A receptor, strongly inhibits tumor growth and liver metastasis of gastric cancer.²⁶

Our results imply that ET-1 stimulates the secretion of LPL from Ehrlich ascites tumor cells by stimulating the PLC-PKC signaling pathway through the ET-A receptor. Finally, since LPL plays an important role in supplying energy and cell components for tumor growth and proliferation, we anticipate that this stimulatory effect of ET-1 promotes tumor growth.

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