Enhancement of the Vanadate-Stimulated Release of Lipoprotein Lipase Activity by Astilbin from the Leaves of Engelhardia Chrysolepis

Toshio Motyashiki,a Mikoto Miyake,a Tetsuo Morita,b Kenji Mizutani,c Hitoshi Masuda,c and Hiroshi Ueki,a

Department of 1st and 2nd Biochemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Fukayama University, 1 Gakuen-cho, Fukayama, Hiroshima 729-02, Japan and Biology and Chemistry Laboratory, Maruzen Pharmaceuticals Co., Ltd. c Sanada, Takamischi-cho, Fukayama, Hiroshima 729-01, Japan.

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Astilbin, a dihydroflavonol rhamnoside isolated from the leaves of Engelhardia chrysolepis, enhanced the vanadate-stimulated release of lipoprotein lipase (LPL) activity from rat isolated fat pads. N-[2-(Methylamino)ethyl]-5-isoquinolinesulphonamide (H-8), a potent inhibitor of cAMP-dependent protein kinase (PKA), markedly inhibited the enhancement by astilbin. Lipolysis in the fat pads was stimulated by astilbin alone in a dose-dependent manner and this stimulation was suppressed in the presence of vanadate, probably due to its antilipolytic action. A significant enhancement by astilbin was observed with increasing effects of vanadate on cAMP content in the fat pads and on cAMP phosphodiesterase (PDE) activity in the particulate fraction although astilbin alone showed only a slight increase in the cellular cAMP content and PDE activity. Astilbin may enhance the vanadate-stimulated release of LPL activity through a synergistic effect on an increase in the cellular cAMP content produced by vanadate accompanied by more potent activation of PKA.

Key words lipoprotein lipase; vanadate; astilbin; cAMP; lipolysis; cAMP phosphodiesterase

The leaves of Engelhardia chrysolepis Hance (Chinese name, huang-qi) have been used from ancient times as a sweet tea to prevent obesity and a folk medicine to treat abdominal pain and fever in China; it is also drunk as a health beverage (Japanese name, Kohki tea) in Japan. Kohki tea contains astilbin, a dihydroflavonol rhamnoside, as a major component, together with isomers as minor ones. Astilbin exhibits a variety of biological actions such as reducing the total liver cholesterol concentration, protecting against oxidative damage to liver mitochondria and hemolysis of erythrocytes, and inhibiting lens aldose reductase to prevent cataract formation in diabetes. We have reported that sodium orthovanadate (vanadate) stimulates the release of lipoprotein lipase (LPL) activity from isolated rat fat pads and increases the LPL activity in the fat pads, and that quercetin, a dehydroflavone, enhances the stimulated release of LPL activity in fat pads produced by vanadate. However, the involvement of astilbin in the regulation of LPL activity produced by vanadate is unknown.

In the present paper, we show that astilbin enhances the vanadate-stimulated release of LPL activity through a synergistic effect on the increase in cAMP content in the fat pads.

MATERIALS AND METHODS

Materials Vanadate (Na₃VO₄) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Bovine serum albumin (Fraction V, free of fatty acids) and N-[2-(methylamino)ethyl]-5-isoquinolinesulphonamide (H-8) were obtained from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan). Intralipos (an emulsion composed of 1.2% yolk lecithin, 2.5% glycerol, and 10% soybean oil) was from Green Cross Co. (Osaka, Japan). Aquasol-2 and [2,8-H]cAMP (1.158 TBq/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). The cAMP enzyme immunoassay system was obtained from Amersham International plc (Little Chalfont, England) and the Bio-Rad protein assay kit was from Nippon Bio-Rad Laboratories, K. K. (Tokyo, Japan). Astilbin, which had been isolated from the leaves of Engelhardia chrysolepis, was kindly supplied by Maruzen Pharmaceutical Co., Ltd. (Fukayama, Japan). All other chemicals were of analytical grade.

Animals Male rats, weighing 200—220 g, were fed a commercial pellet diet ad libitum, given free access to water for 1 week in an air-conditioned animal facility, and starved for 24 h before each experiment.

Preparation of Fat Pads and Incubation with Vanadate Epididymal adipose tissues were quickly removed from rats killed under ether anesthesia and cut into small pieces (30—40 mg) with scissors in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 5 mM glucose and 2% bovine serum albumin (KRBGA) at 37 °C. The stock solution of vanadate (100 mM, pH 7.4) was diluted with deionized water to the desired concentrations immediately before use. The fat pads (1 g) were preincubated with or without astilbin and further incubated with or without vanadate in 10 ml KRBGA at 37 °C for the indicated periods and washed three times with physiological saline at 4 °C, as described previously.

Determination of LPL Activity To determine the stimulated release of LPL activity by vanadate, the incubated media were used as enzyme solutions. Intralipos was activated by incubation with an identical volume of heated rat serum at 37 °C for 30 min. The activated Intralipos (0.05 ml) was added to a mixture of 0.2 ml of the enzyme solution and 0.25 ml 30 mM Tris–HCl buffer, pH 8.5, containing 2% bovine serum albumin in the presence or absence of 1 mM NaCl and incubated at 37 °C for 30 min. The free fatty acids (FFA) produced were determined by the method of Duncombe. The LPL activity was calculated from the difference between the presence and absence of 1 mM NaCl and expressed as μmol FFA produced/h/g fat pads.

Determination of Lipolysis FFA in the incubated media (0.5 ml), which was used to assay the LPL activity, was determined by the method of Duncombe.
Determination of cAMP Phosphodiesterase (PDE) Activity 

The incubated fat pads (1 g) were homogenized in a Potter-Elvehjem homogenizer in 2 ml 10 mm Tris–HCl buffer, pH 7.4, containing 0.25 μm sucrose and protease inhibitors (1 mm benzamidine and 0.1 mm p-amidino-phenylmethane-sulfonyl fluoride). After centrifugation at 1000×g for 10 min, the infranatant was further centrifuged at 12000 and 17500×g for 4.5 min each to remove the mitochondrial fraction and at 105000×g for 60 min to separate the particulate fraction.11 The PDE activity of the particulate fraction was determined by a slight modification of the method of Kono et al.11,12 An aliquot (0.05 ml) of enzyme solution was incubated with 250 nm [2,8-3H]cAMP (0.9 KBoq) in 33 mm Tris–HCl buffer, pH 7.4, containing 4 mm MgCl2 at 30 °C for 20 min, in a total volume of 0.25 ml. The incubation was terminated by the addition of 0.1 ml 0.1 N HCl. A mixture (0.05 ml) of 5 mm AMP and cAMP was added to the incubation mixture, allowed to stand at 70 °C for 4 min, chilled, and neutralized with 0.1 ml 0.1 N NaOH. After the addition of 50 μg snake venom in 0.05 ml 0.1 n Tris–HCl buffer, pH 8.0, to the neutralized solution, the incubation was carried out at 37 °C for 20 min and terminated by the addition of a mixture (0.05 ml, pH 7.0) of 200 mm EDTA and 5 mm adenosine. The reaction mixture (0.05 ml) was applied to a 5.5 × 30 mm column of Dowex 1 × 8 (200–400 mesh in chloride form). The loaded column was eluted with water. The first 1.4 ml of the effluent was discarded, and the next 2.5 ml was collected. The adenosine radioactivity in the 2.5 ml fraction was determined in Aquasol-2 using an Aloka liquid scintillation counter (LSC-700). Protein concentrations were determined using a Bio-Rad protein assay kit. The PDE activity was expressed as pmol cAMP hydrolyzed/min/mg protein.

Determination of cAMP Content in Fat Pads 
The fat pads (200 mg) were preincubated with or without astilbin for 15 min and further incubated with or without vanadate for 3 min.8 The incubated fat pads were quickly separated, frozen in dry ice-acetone to terminate the reaction, and homogenized in 1 ml chilled 6% trichloroacetic acid (TCA) for 1 min using a Niton Medical and Scientific homogenizer (Physocron, NS-310E). After centrifugation at 1500×g for 10 min, the infranatant was extracted four times with chilled H2O-saturated diethyl ether to remove TCA. The cAMP content in the residual solution was determined using a commercially available cAMP enzyme immunoassay system.

Statistical Analysis 
All results are presented as the mean±S.E. of four observations. Similar results were obtained in at least two separate experiments. The data were analyzed by Student’s t-test.

RESULTS AND DISCUSSION

Figure 1 shows the effect of astilbin on the stimulated release of LPL activity by vanadate. When the fat pads were preincubated with astilbin over a concentration range up to 400 μM, a significant enhancement of the effect of vanadate (1 mm) was observed with 100–400 μM astilbin. Astilbin alone did not have any effect on the release of LPL activity from fat pads. We have already reported that vanadate stimulates the release of LPL activity from the fat pads through a mechanism involving the activation of cAMP-dependent protein kinase (PKA) due to a rapid increase in cellular cAMP content.8 Therefore, the effect of H-8, a potent inhibitor of PKA,13 on the enhancement by astilbin was investigated. When the fat pads were preincubated with astilbin in the presence of H-8, a significant inhibition of the enhancement by astilbin was observed at 100 and 500 μM H-8 (Fig. 2). Lipolysis in the fat pads was stimulated by astilbin alone in a dose-dependent manner and this stimulation was suppressed in the presence of vanadate (Fig. 3). Although vanadate stimulates cAMP production, it antagonizes lipolysis mediated by isoproterenol and ultimately has an antilipolytic effect.8,14 The exact mechanism(s) by which vanadate, as well as insulin, antagonizes lipolysis is still unknown. However, one possible explanation is that the antilipolytic action may involve a phosphorylation cascade stimulated by inhibition of membranal protein tyrosine phosphatase due to vanadate.14 Therefore, the lipolysis of astilbin may also be antagonized in a similar manner to the antilipolytic action on isoproterenol. The cellular cAMP content is mainly maintained by
Fig. 3. Effect of Astilbin on Lipolysis in Fat Pads in the Presence or Absence of Vanadate.

The fat pads were preincubated with astilbin at the indicated concentrations for 15 min and further incubated with (*) or without (○) vanadate (1 mM) for 90 min. Significant differences compared with the group without astilbin or vanadate: *p<0.05 and **p<0.01.

Fig. 4. Effect of Astilbin on Increase in Cellular cAMP Content by Vanadate.

The fat pads were preincubated with or without astilbin (200 μM) for 15 min and further incubated with or without vanadate (1 mM) for 3 min. Significant differences compared with the vanadate-treated group without astilbin: *p<0.05.

a balance of adenylate cyclase and PDE activity, both of which produce cAMP from ATP and then hydrolyze it to 5'-AMP. Of the various PDE isozymes, the cGMP-inhibited PDE (type III) activity, which is found in the particulate fraction of adipose tissues, was stimulated by vanadate as well as insulin. Therefore, the effects of astilbin on the regulation of cellular cAMP content by vanadate were investigated. Enhancement of the vanadate-increased cAMP content in the fat pads was observed with 200 μM astilbin (Fig. 4). The vanadate-stimulated PDE activity was also enhanced by astilbin at the same concentration (Fig. 5). Astilbin alone resulted in a slight increase in the cAMP content and PDE activity although quercetin had a more potent β-adrenergic action. We have reported that the incubation of fat pads with vanadate in the presence of 1 mM 3-isobutyl-1-methylxanthine or 2 mM dibutyryl cAMP completely inhibits an increase in LPL activity in the fat pads produced by vanadate. In cultured avian adipocytes, incubation with 0.5 mM dibutyryl cAMP resulted in both a decreased cellular LPL content and a diminished rate of enzyme secretion. To stimulate the release of LPL activity by vanadate, a transient increase in the cellular cAMP content, based on a combination of a rapid activation of adenylate cyclase and sequential activation of PDE to reduce the elevated cAMP level, seems to be necessary.

In conclusion, Astilbin seems to enhance the vanadate-stimulated release of LPL activity through a synergistic effect on the increase in cellular cAMP content produced by vanadate accompanied by a more potent activation of PKA.

REFERENCES