Protein Kinase Inhibitor H-7 Increases Lipoprotein Lipase Activity in Isolated Rat Fat Pads

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A protein kinase inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7) increased lipoprotein lipase (LPL) activity in isolated rat fat pads in a time- and dose-dependent manner. The incubation of H-7 with partially purified LPL did not affect its activity. Under the marked inhibition of protein synthesis by cycloheximide, H-7 still showed a full effect on the increase in LPL activity. A slight but significant increase in LPL activity in the fat pads was observed with inhibitors of cyclic nucleotide-dependent protein kinase. H-7, therefore, may increase LPL activity through processes other than the direct activation of the LPL molecule, or the stimulation of LPL molecule synthesis; probably through a decrease in the activity of protein kinases, especially protein kinase C.

Keywords H-7; protein kinase inhibitor; protein kinase C; lipoprotein lipase; adipose tissue

Introduction

Lipoprotein lipase (LPL, EC 3.1.1.34), which is localized on the membrane surface or the vascular endothelium in extrahepatic tissues, plays an important role in the metabolism of triacylglycerol-rich lipoproteins, including chylomicron and very low density lipoprotein (VLDL).^{1–4)} 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7) is known to be a most potent inhibitor of protein kinase C (PKC) involved in the transduction of biological signals.⁵⁾ H-7 has been used to determine whether or not PKC is involved in various cellular responses in many experiments. Recently it has been reported that H-7 causes a depletion of normal active microfilament bundles in 3T3 cells,⁶⁾ and that H-7 potentiates the release of unsaturated fatty acids, such as oleic, linoleic and arachidonic acids, in A23187-stimulated neutrophils.⁷⁾ These reports show that H-7 appears to have effects in addition to inhibiting the response of PKC. It remains unclear whether the regulation of protein synthesis is involved in a part of the action of H-7. There are only a few reports on the relationship between LPL activity and PKC activity.

In this report, we show that H-7 stimulates LPL activity in rat fat pads without the elevation of protein synthesis.

Materials and Methods

Materials L-[3,4,5-³H(N)]Leucine (5.31 TBq/mmol) was from New England Nuclear (Boston, MA). Cycloheximide was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Protein kinase inhibitors (H-7, H-8, and HA1004), which are derivatives of isoquinolinesulfonamide³⁾ were purchased from Seikagaku Industries, Ltd. (Tokyo, Japan). Staurosporine⁸⁾ was a gift from Dr. Matsuda (Kyowa Hakko Kogyo Co., Ltd., Tokyo). All other chemicals were of analytical grade.

Animals Male Wistar rats (200–220 g) were fed on a commercial laboratory chow *ad libitum* for a week and starved for 24 h before each experiment.

Preparation of Fat Pads and Enzyme Assay As described previously,⁹⁾ the fat pads, prepared from epididymal adipose tissue, were incubated with various agents at 37°C for indicated periods in 2 ml of Krebs–Ringer bicarbonate buffer containing 5 mM glucose and 2% bovine serum albumin. After incubation, the fat pads were homogenized on 30 mM Tris–HCl buffer, pH 8.5, 4°C and the resultant supernatant was used as the enzyme preparation of LPL⁹⁾ and assayed using the colorimetric method.^{10,11)}

Results and Discussion

A linear increase in LPL activity was observed with the incubation of the fat pads with H-7 at a concentration of 100 μ M for up to 180 min (Fig. 1). When the fat pads were incubated for 120 min with H-7 over a concentration range

of 0–200 μ M, LPL activity in the fat pads increased in a dose-dependent manner up to 200 μ M (Fig. 2). To determine whether the direct action of H-7 on the LPL molecule is involved in the increase in LPL activity, the enzyme fraction was obtained from homogenized fat pads, as described in Materials and Methods, or prepared to partial purification by affinity chromatography on a heparin–Sephacrose column.¹²⁾ The LPL activity of both fractions was never elevated by the addition of H-7 (data not shown). Fig-

Fig. 1. Time Course of Increase in LPL Activity in the Fat Pads by H-7

The fat pads were incubated with H-7 (100 μ M, ●) or without (○) for 0–180 min. The LPL activity in the fat pads in each case was determined.

Fig. 2. Dose-Response Curve of the Increase in LPL Activity in Fat Pads by H-7

After the fat pads were incubated for 120 min at the indicated concentration of H-7, LPL activity in the fat pads was determined.

Fig. 3. Effect of Cycloheximide on Incorporation of [^3H]Leucine into the Protein of the Fat Pads Incubated with or without H-7

The fat pads were incubated with H-7 (100 μM , ●) or without (○) for 120 min at the indicated concentrations of cycloheximide under the addition of [^3H]leucine (18.5 kBq).^{9,13} The incorporation of radioactivity into the protein in the fat pads was determined.

Fig. 4. Effect of Cycloheximide on H-7 Induced Increases in LPL Activity in Fat Pads

The fat pads were incubated with H-7 (100 μM , hatched bar) or without (white bar) for 120 min at the indicated concentrations of cycloheximide. LPL activity in the fat pads was determined.

Figure 3 shows the inhibitory profiles by cycloheximide of [^3H]leucine into the protein of the fat pads. In the H-7-treated fat pads, the incorporation of [^3H]leucine into protein was reduced to 62% of the control by H-7, and was further decreased with an increase in the concentration of cycloheximide, similarly to non-treated fat pads. Although the protein synthesis was inhibited to 59 and 20% of the original level by 0.1 and 1 μM cycloheximide, respectively, the increase in LPL activity by H-7 was never inhibited (Fig. 4). These results show that the H-7-stimulated increase in LPL activity is dissociated from the stimulation of protein synthesis. The increase in LPL activity in fat pads by various protein kinase inhibitors is shown in Fig. 5. LPL activity in the fat pads was increased 1.5-fold by staurosporine, a PKC inhibitor different from isoquinolinesulfonamides.⁸⁾ A

Fig. 5. Effect of Various Protein Kinase Inhibitors on LPL Activity in the Fat Pads

The fat pads were incubated with a protein kinase inhibitor, that is, H-8 (100 μM), H-7 (100 μM), HA1004 (100 μM), staurosporine (100 nM) or without. LPL activity in the fat pads was determined. Significantly different from no inhibitor at $p < 0.01$ (a) and $p < 0.05$ (b).

slight but significant increase in LPL activity was also observed with HA1004 and H-8, inhibitors of cyclic nucleotide-dependent protein kinases.³⁾

In conclusion, H-7 may increase the LPL activity through processes which involve neither direct activation of the LPL molecule, nor stimulation of LPL molecule synthesis; probably, the phosphorylation-dephosphorylation system including PKC is involved in the regulation of LPL activity.

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