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Inhibition of hormone-sensitive lipase by intermediary lipid metabolites

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Hormone-sensitive lipase (HSL) is inhibited in a non-competitive manner by oleoyl CoA, oleic acid and 2-monopalmitoylglycerol, 50% inhibition being observed at concentrations of approx. 0.1 μM, 0.5 μM and 500 μM, respectively. HSL is a key enzyme in lipid metabolism, mobilising triacylglycerol and cholesterol ester stores in several tissues. Feedback inhibition of HSL by oleoyl CoA and oleic acid may therefore prevent accumulation of free fatty acids and cholesterol in the cell, whereas 2-monoacylglycerol may act as a feedback inhibitor if the capacity of monoacylglycerol lipase is exceeded.

Hormone-sensitive lipase; Oleic acid; Oleoyl CoA; 2-Monopalmitoylglycerol; Feedback inhibition

1. INTRODUCTION

Hormone-sensitive lipase (HSL) is responsible for the hydrolysis of triacylglycerols and cholesterol esters in several tissues (reviewed in [1]). It catalyses the rate-limiting step in lipolysis in adipose tissue [2] and cardiomyocytes [3], whereas in adrenal cortex [4,5] and macrophages [6] its role is apparently to mobilise cholesterol from esterified stores for steroidogenesis or excretion from the cell, respectively. The only known mechanism by which the activity of HSL is regulated is by reversible phosphorylation of the enzyme protein; phosphorylation of the 'regulatory' site (site 1) of HSL by cyclic AMP-dependent protein kinase results in activation of the enzyme [2,7]. HSL also has a 'basal' phosphorylation site (site 2) which is acted on by several protein kinases including the AMP-activated kinase [8,9]. It appears that phosphorylation of these two sites is mutually exclusive; therefore if the 'basal' site is phosphorylated then the 'regulatory' site is inaccessible to phosphorylation by the cyclic AMP-dependent protein kinase and the enzyme cannot be activated [9].

Here we have demonstrated an additional, physiologically relevant, mode of regulation of HSL, namely inhibition of the enzyme by oleoyl CoA, oleic acid and 2-monopalmitoylglycerol. Two other enzymes of lipid metabolism, involved in fatty acid synthesis (acetyl CoA carboxylase) and cholesterol synthesis (HMG-CoA reductase), are also subject to inhibition by oleoyl CoA and oleic acid, both directly [10,11] and via phosphorylation by the AMP-activated kinase, which is itself activated by an acyl CoA-dependent kinase [12]. A feedback control mechanism may therefore exist to regulate the tissue levels of free fatty acids and cholesterol via the co-ordinated regulation of the three enzymes mentioned above.

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Abbreviations: HSL, hormone-sensitive lipase; CoA, coenzyme A; HMG-CoA, hydroxymethylglutaryl-CoA; PNPB, *p*-nitrophenylbutyrate; CMC, critical micellar concentration; PDH, pyruvate dehydrogenase.

2. MATERIALS AND METHODS

2.1. *Materials*

HSL was prepared from bovine perirenal adipose tissue as in [13]. Sources of lipid substrate, protease inhibitors and the non-ionic detergent $C_{13}E_{12}$ were as described in [13]. Co-enzyme A (CoA), oleoyl CoA, oleic acid, 2-monopalmitoylglycerol, cholesterol and *p*-nitrophenylbutyrate were from Sigma.

2.2. *Enzyme activity*

HSL activity was assayed against cholesterol [^{3}H]oleate at pH 7.0 in ethanolic suspension essentially as described in [14], except that BSA was omitted from the substrate mix. One unit of enzyme activity catalyses the release of 1 μmol oleic acid per min. Activity against the water soluble substrate *p*-nitrophenylbutyrate (PNPB) was assayed as described in [15], assay mixtures consisting of the appropriate concentration of PNPB dissolved in 1% acetonitrile in 10 mM HEPES, pH 7.0, 10% (v/v) glycerol, 10 mM NaCl, 1 mM DTT. One unit of enzyme activity catalyses the release of 1 μmol of *p*-nitrophenol per minute. Effectors were added to enzyme immediately prior to assaying activity; water-insoluble lipids were dissolved either in 0.025 vol. ethanol (for cholesterol esterase assay), or 0.02 vol. acetonitrile (for assay against PNPB). Controls in the absence of inhibitor were performed in the presence of the appropriate solvent.

3. RESULTS AND DISCUSSION

HSL activity against cholesterol oleate and PNPB was found to be inhibited by oleoyl CoA, oleic acid and 2-monopalmitoylglycerol (Fig. 1). In contrast, other HSL reaction products, namely cholesterol and glyc-

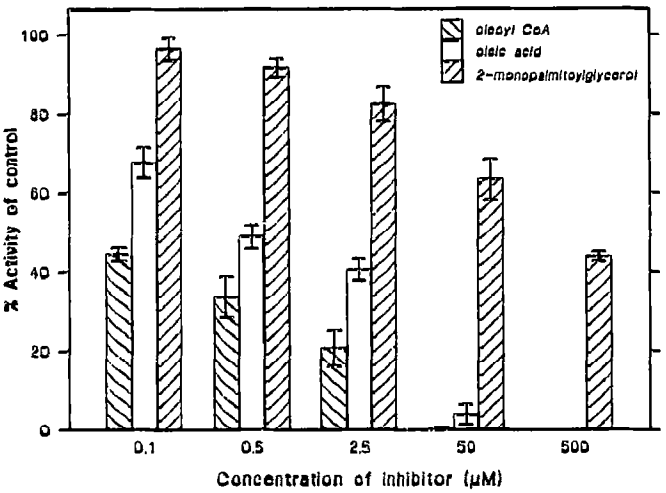

Fig. 1. Inhibition of HSL by oleoyl CoA, oleic acid and 2-monopalmitoylglycerol. Inhibitors were added to HSL (in 0.025 vol. ethanol) to give the final concentrations indicated and enzyme immediately assayed against cholesterol [^{3}H]oleate as described in Section 2. Activities are expressed as a percentage of controls observed in the presence of 0.025 vol. ethanol alone, and are mean values ± S.E.M. (n = 4).

erol, had no effect on enzyme activity (Table I). As can be seen in Fig. 2, oleoyl CoA inhibited the cholesterol esterase activity of HSL in a non-competitive manner; this mode of inhibition was also observed with oleic acid and 2-monopalmitoylglycerol (results not shown). Similar results were obtained for inhibition of the PNPB hydrolysing activity of HSL (Fig. 3), suggesting that this water-soluble substrate is hydrolysed at the same site as the lipid substrate. This was further supported by the observation that cholesterol oleate inhibited PNPB hydrolysis by HSL in a competitive manner (Fig. 4).

Of the inhibitors examined, oleoyl CoA was found to have the greatest effect, decreasing HSL activity by 50% at approximately 0.1 μM (Fig. 1). Oleic acid resulted in 50% inhibition of HSL at approximately 0.5 μM, whereas approximately 500 μM of 2-monopalmitoylglycerol was required for a similar inhibition (Fig. 1). The greater inhibition induced by oleoyl CoA cannot be solely attributed to the CoA moiety, as CoA alone was found to have no effect on the activity of HSL (Table I); other workers have reported that both the 3′-phosphate moiety of CoA and the long-chain acyl residue are essential for inhibition of acetyl CoA carboxylase by fatty acyl CoAs [16].

The inhibition of HSL by oleoyl CoA and oleic acid was not due to any detergent effects of these compounds, as inhibition was observed at concentrations well below their critical micellar concentrations (CMC); the reported CMC for fatty acyl CoAs is 30–60 μM [17] and for fatty acids is 0.8–1.0 mM [18]. This implies that inhibition of HSL by oleoyl CoA and oleic acid is via binding to a specific site on the enzyme protein.

Fatty acids have been reported to inhibit adenylate cyclase and therefore cyclic AMP accumulation in the adipocyte [19]. This feedback would reduce HSL activation by phosphorylation; however, direct inhibition of HSL activity may also result from elevated levels of fatty acids in the cell. These two inhibitory mechanisms may therefore act together to prevent further accumulation of fatty acids released from lipolysis and therefore possible tissue damage via their detergent properties.

The observed inhibition of HSL by 2-monopalmitoylglycerol (Fig. 1) may also be of significance as a feedback control of lipolysis. As a result of preferential cleavage of 1(3)-ester bonds by HSL, the hydrolysis of

Table I

Effect of cholesterol, glycerol and CoA on HSL activity

	Cholesterol esterase activity (U/ml)	PNPB hydrolase activity (U/ml)
Control	1.04 ± 0.005	4.52 ± 0.02
Cholesterol		
5.0 μM	1.06 ± 0.005	4.50 ± 0.005
500 μM	1.08 ± 0.01	4.49 ± 0.01
Glycerol		
5.0 μM	1.03 ± 0.01	4.46 ± 0.01
500 μM	1.05 ± 0.015	4.50 ± 0.03
CoA		
5.0 μM	1.02 ± 0.005	4.48 ± 0.015
500 μM	1.04 ± 0.01	4.49 ± 0.02

Effectors were added to HSL to give the final concentrations shown and resultant enzyme activity against either cholesterol [^{3}H]oleate or PNPB assayed as described in Section 2. Activities are expressed as mean values ± S.E.M. (n = 4).

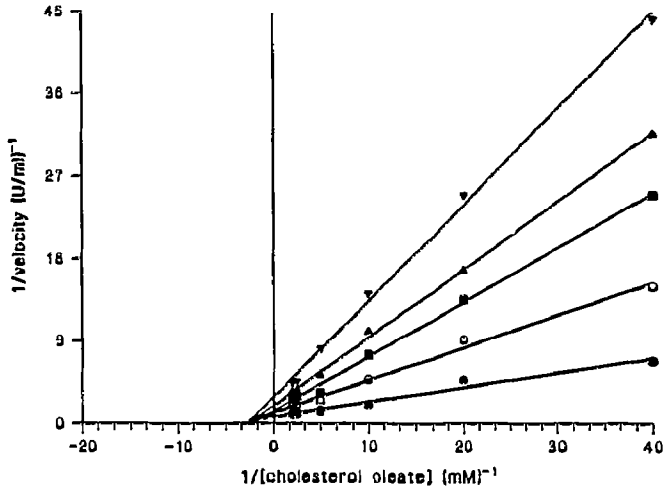

Fig. 2. Lineweaver-Burke analysis of the inhibition of HSL cholesterol esterase activity by oleoyl CoA. HSL activity was assayed against cholesterol [^{3}H]oleate as described in Section 2, either in the absence of oleoyl CoA (●) or in the presence of 1.0 μM (○), 2.5 μM (■), 10 μM (▲) or 50 μM (▼) oleoyl CoA. A constant specific radioactivity of cholesterol [^{3}H]oleate was maintained at all concentrations of the substrate used (approx. 2000 dpm/nmol).

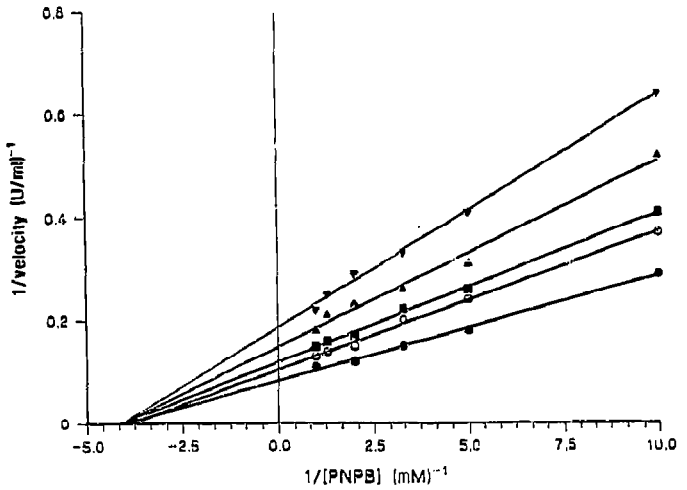

Fig. 3. Lineweaver-Burke analysis of the inhibition of HSL activity against PNPB by oleoyl CoA. HSL was assayed against PNPB as described in Section 2, either in the absence of oleoyl CoA (○) or in the presence of 1.0 μM (●), 2.5 μM (■), 10 μM (▲) or 50 μM (▼) oleoyl CoA. Assays were performed at 30°C for 20 s, with product formation measured by absorbance at 400 nm.

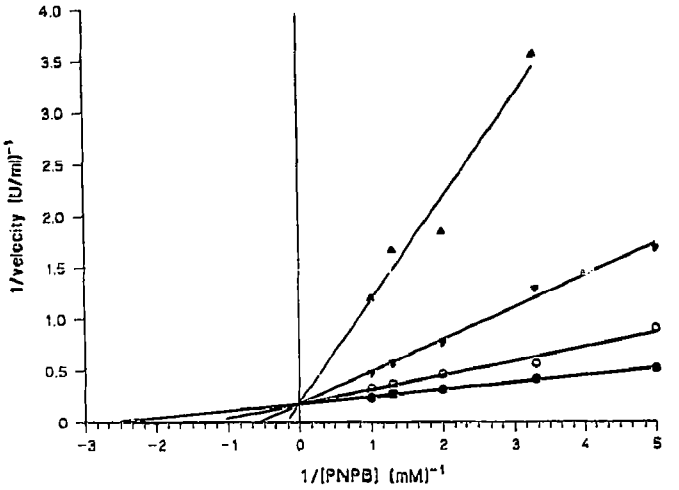

Fig. 4. Lineweaver-Burke analysis of the inhibition of HSL activity against PNPB by cholesterol oleate. HSL activity was assayed against PNPB as in the legend to Fig. 3, either in the absence of cholesterol oleate (●) or in the presence of 1.0 μM (○), 10 μM (▼) or 50 μM (▲) cholesterol oleate.

diacylglycerol yields 2-monoacylglycerol. This is a poor substrate for HSL and therefore a distinct 2-monoacylglycerol lipase is required for the final step of lipolysis to yield free fatty acids and glycerol [20]. If the rate of production of 2-monoacylglycerol by HSL exceeds its rate of hydrolysis by the 2-monoacylglycerol lipase, the resultant accumulation of the lipid may prevent its further generation. However, the physiological conditions under which this might occur are unknown as it has been reported that the 2-monoacylglycerol lipase is present in adipocytes in sufficient quantity that it is not rate-limiting, even during hormonally-stimulated lipolysis [20].

The inhibition of HSL by fatty acyl CoAs may have a role in cardiac muscle where, under normal conditions, endogenous triacylglycerol is the major energy store [21]. However, under conditions of hypoxia and ischaemia, decreased coronary blood flow and oxygen consumption result in reduced fatty acid oxidation and therefore levels of fatty acyl CoAs rise [22]; the cytosolic concentration of fatty acyl CoAs can rise to approximately 50 μM under such conditions which, as Fig. 1 shows, is sufficient to almost completely inhibit HSL activity. Release of fatty acids from hydrolysis of triacylglycerol stores in the cardiomyocyte will therefore be significantly reduced, preventing further accumulation. Under such conditions the energy metabolism in the heart changes and stored glycogen becomes the major source of endogenous energy, with glucose being metabolised anaerobically to provide energy for cardiac function [21].

Two key enzymes in fatty acid synthesis (acetyl CoA carboxylase) and cholesterol synthesis (HMG-CoA reductase) are also inhibited directly by oleic acid and oleoyl CoA in a non-competitive manner and over similar concentration ranges as observed here for HSL [10,11]. All three enzymes are phosphorylated by the AMP-activated kinase; phosphorylation of acetyl CoA carboxylase and HMG-CoA reductase results in direct inhibition of these enzymes [12], whereas phosphorylation of HSL by the AMP-activated kinase blocks subsequent phosphorylation and activation by cyclic AMP-dependent protein kinase [9]. The AMP-activated kinase is itself activated by phosphorylation by a distinct 'kinase-kinase' which is activated by nanomolar concentrations of fatty acyl CoA [12]. Thus a co-ordinated feedback mechanism may exist whereby elevated levels of fatty acids or fatty acyl CoAs regulate the levels of free cholesterol and fatty acids in the cell via inhibition of their synthesis or mobilisation from stores. This mechanism may come into effect without any detectable changes in the total intracellular concentration of the inhibitors due to their compartmentalisation, which may be effected by the fatty acid binding protein [11]. This 'double' feedback mechanism of direct enzyme inhibition and activation of an inhibitory kinase is also found in other systems. For example, NADH and acetyl CoA, end-products of pyruvate dehydrogenase (PDH), inhibit its activity both directly and via activation of PDH-kinase (reviewed in [23]).

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