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Autolysis of isolated adipocytes by endogenously produced fatty acids

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Isolated rat adipocytes, in which lipolysis was maximally stimulated by isoproterenol, continued to produce fatty acids in excess of the high-affinity binding capacity of the fatty acid acceptor bovine serum albumin in the incubation medium. At an average of 17 mol fatty acids/mol serum albumin, there was a burst of lactate dehydrogenase activity recovered in the incubation medium, indicating cell lysis. It is concluded that endogenously produced fatty acids will cause autolysis of adipocytes in vitro. Actively lipolysing adipocytes were more fragile than resting cells, since increasing amounts of lactate dehydrogenase activity was recovered in the medium during active lipolysis.

Adipocyte; Lipolysis; Fatty acid; Serum albumin

1. INTRODUCTION

Fatty acids are produced in the adipose tissue and released into the bloodstream. In the blood, serum albumin binds the fatty acids and acts as a vehicle for transport to metabolizing tissues. Free fatty acids in their dissociated form are amphiphilic molecules that will act as a detergent in solution. Serum albumin has 7 high-affinity binding sites for palmitic acid [1–3], with dissociation constants ranging from 10^{-8} to 10^{-5} M [2]. With normally less than 1 mol fatty acid bound/mol albumin [4], the free concentration of fatty acids in the blood is below micromolar. If the capacity for serum albumin binding of fatty acids is exceeded, the rate of adipose tissue lipolysis is thought to be reduced through product inhibition of the hormone-sensitive lipase [5] and perhaps also fatty acid inhibition of the adenylate cyclase [5], which governs the lipase activity through cAMP [6].

Isolated adipocytes in suspension [7] is a popular system in which to study effects of hormones. Lipolysis is very sensitive to stimulation by cAMP-raising hormones and to inhibition by insulin [8]. In this model system, the adipocytes are usually incubated in medium containing defatted bovine serum albumin as the extracellular fatty acid acceptor [7].

In this report, I describe how isolated adipocytes that are incubated in the presence of the β-adrenergic agonist isoproterenol, will continue to produce fatty acids in excess of the capacity of the albumin to bind the fatty acids produced. It is demonstrated that above 7 mol fatty acids/mol albumin in the incubation medium, the cytosolic marker protein lactate dehydrogenase is rapidly and almost completely recovered in the medium, indicating cell lysis.

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2. MATERIALS AND METHODS

Rat adipocytes (135 g Sprague–Dawley rats, Alab, Sweden, starved overnight and killed by cervical dislocation) were prepared by collagenase digestion in the presence of 0.2 μM adenosine (Boehringer) [9]. Cells (30 μl packed cell-volume/ml, ca. 5×10^5 cells/ml) were incubated with magnetic stirring in Krebs-Ringer solution (0.13 M NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4) containing 5 mM glucose, 0.1 μM phenylisopropyladenosine (a non-metabolized adenosine analog that blocks non-stimulated lipolysis [9], from Boehringer), 1 U/ml adenosine deaminase (Boehringer) and 20 mg/ml defatted [10] bovine serum albumin (Sigma). The incubation was kept at pH 7.40 by a pH-state [11]. After a 90 min preincubation, 1 μM D,L-isoproterenol (Sigma) was added at time zero. The fatty acid release was continuously recorded by pH-stat titration of the cell incubation [11]. At the indicated time points, samples were withdrawn and cells immediately separated from the medium by centrifugal flotation for 3 s (Beckman microfuge). The medium was cooled in an aluminum block kept on ice and the activity of lactate dehydrogenase was determined [12] within 2 h.

3. RESULTS AND DISCUSSION

A time course of fatty acid release from isolated rat adipocytes incubated with isoproterenol is shown in Fig. 1. Also shown is the recovery of lactate dehydrogenase activity in the medium. The starting level of lactate dehydrogenase activity indicates that some cells have been broken during the 90 min preincubation before isoproterenol addition. The presence of phenylisopropyladenosine, a non-metabolized adenosine-receptor agonist, in the incubation medium maintains lipolysis near zero in the absence of isoproterenol. The rate of lactate dehydrogenase leakage into the medium increases when the fatty acid

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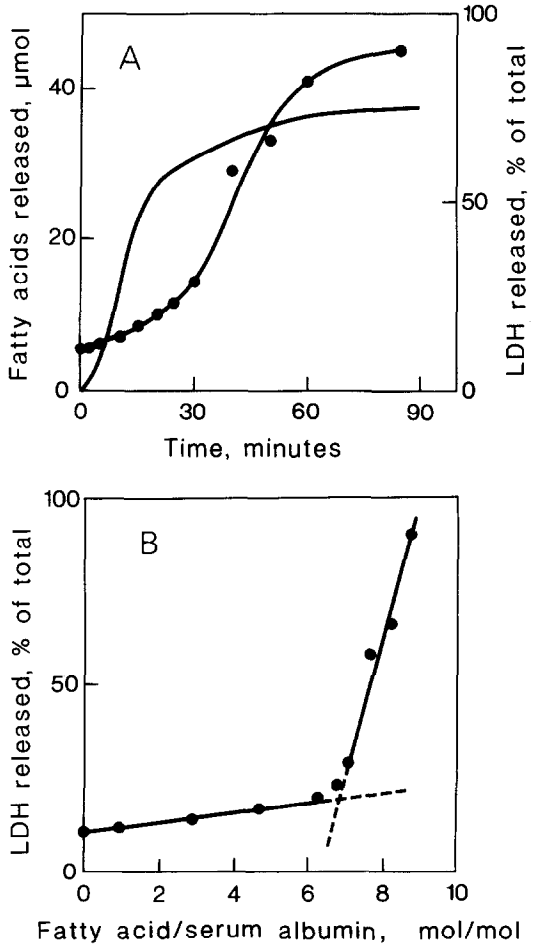

Fig. 1. Release of fatty acids and lactate dehydrogenase (LDH) from adipocytes. (A) Time course. Fatty acids released (——); LDH released (•). (B) Dependence of lactate dehydrogenase released on the average fatty acid:serum albumin ratio. The concentration of bovine serum albumin was determined spectrophotometrically: $A^{1\%}_{280nm}$ = 6.5. The total activity of lactate dehydrogenase in a cell homogenate was 370 nmol/min per ml.

release is stimulated by isoproterenol (Fig. 1), probably indicating that the steady-state level of fatty acids partitioned in the cell membranes during active lipolysis makes the cells more brittle (cf. [9]). After 30 min of stimulated lipolysis, when the fatty acid release has already slowed down, the amount of lactate dehydrogenase leakage increases dramatically (Fig. 1A).

The relationship between the average fatty acid:serum albumin ratio and the lactate dehydrogenase activity recovered in the incubation medium is depicted in Fig. 1B. At low fatty acid:serum albumin ratios, there is a continuous and small increase of lactate dehydrogenase activity in the medium. The cells clearly produce fatty acids in excess of 7 mol/mol serum albumin, when there is a burst of lactate dehydrogenase released into the medium.

These findings have demonstrated that adipocyte lipolysis will continue to produce fatty acids well in excess of the high-affinity binding capacity of serum albumin for fatty acids, albeit at a considerably reduced rate. Due to their amphiphilic character, the free fatty acids will cause cell lysis. If precautions are not taken to limit the production of free fatty acids in an experimental setup, the inevitably ensuing autolysis of the adipocytes will cause erroneous results. This is especially pertinent when the release of other substances from the adipocytes are studied (cf. [13]). The important parameters to be controlled are the rate of lipolysis, the cell concentration, the serum albumin concentration and the length of incubation. Stimulated cells incubated at a high concentration will rapidly exhaust the serum albumin.

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