PREPARATION OF ADRENALINE-SENSITIVE LIPID MICELLES FROM RAT EPIDIDYMAL ADIPOSE TISSUE

by

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SUMMARY. Lipid micelles were prepared from rat epididymal adipose tissue. The micelles were mainly composed of triglyceride with small amounts of protein, sugar, phospholipid, choresterol and choresterol ester.
Adrenaline stimulated lipolysis in intact micelles, but not when they were homogenized.

The lipolytic process in adipose tissue plays a key role in the energy metabolism of the whole animal. By regulating the extent of lipolysis in adipose tissue, a variety of hormones exert important influences on the supply of free fatty acids available as fuel for the muscles and other tissues of the body. Rodbell obtained a homogeneous preparation of fat cells and showed that adrenaline stimulated lipolysis in these cells (1).

It is believed that adrenaline induces lipolysis by stimulating adenyl cyclase. This enzyme catalyzes the formation of cyclic AMP which in turn activates hormone-sensitive lipase (2, 3, 4).

Rodbell prepared plasma membranes from isolated fat cells and found that they contained most of the adenyl cyclase in the cells (5). He disrupted fat cells in hypotonic medium and then obtained the plasma membranes by centrifugation of the disrupted cell suspension.

During the reexamination of Rodbell's work, we found that the lipid micelles discharged from fat cells responded to adrenaline, and this paper gives details of our findings.

MATERIALS AND METHODS

Fat cells were isolated from epididymal adipose tissue of Wistar King rats (150 to 170 g) by the method of Rodbell (1)

Preparation of lipid micelles: The isolated fat cells were suspended in 5 mM Tris-HCl buffer (pH 7.4) (fat cells equivalent to 400 mg adipose tissue per ml).

The suspension was mixed by slowly inverting the centrifuge tube several times and then centrifuged at 200 ×g for 3 min at room temperature. This resulted in formation of three layer, a fat layer, supernatant and pellet fraction. The supernatant fraction was withdrawn through a hypodemic needle and replaced by an equal volume of 5 mM Tris-HCl buffer (pH 7.4). The procedure of mixing by inversion and centrifugation was repeated four times more. Finally the fat layer was suspended in 5 mM Tris-HCl buffer (pH 7.4, fat layer equivalent to 400 mg adipose tissue per ml). This suspension is referred to as lipid micelles.

α-Glycerophosphate dehydrogenase assay: The reaction mixture consisted of 125 µmoles of α-glycerophosphate, 0.8 µmoles of NAD, 50 µmoles of $MgCl_2$, 20 µmoles of Tris-HCl, pH 8.6 and 0.2 ml of enzyme solution in a final volume of 1.0 ml.

Incubation was carried out at 37° for 20 minutes and then 0.2 ml of 10 % Na_2WO_4 and 2 ml of ethanol were added. The mixture was stood for 40 minutes at room temperature and then centrifuged for 10 minutes at 3000 rpm. The optical density of the resultant supernatant at 340 nm was estimated. Activity is expressed as the amount of NADH formed per g of tissue.

Lipase assay: Reaction mixture consisting of 50 µl of a 1:4 dilution of the original solution of Ediol, 500 µl of the enzyme solution and 450 µl of 0.06 M phosphate buffer (pH 7.4) containing 5 % albumin was incubated at 37° for 60 minutes. Then Dole's extraction mixture was added and free acids were assayed by the method of Dole (6).

Palmitic acid was used as a standard.

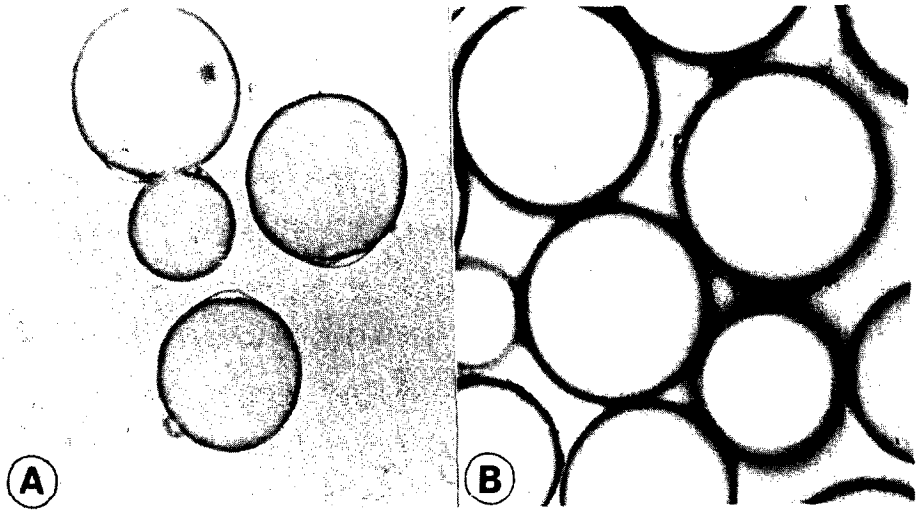

Fig. 1 Photomicrographs of free fat cells and lipid micelles from rat epididymal adipose tissue.
A. fat cells (×400). B, lipid micelles (×400).

Esterase assay: Reaction mixture consisting of 50 µl of 1 M methyl butyrate solution in the ethanol, 500 µl of enzyme solution and 450 µl of 0.06 M phosphate buffer (pH 7.4) was incubated at 37° for 60 minutes. Then Dole's extraction mixture was added and butyric acid released was estimated by the method of Dole (6). Butyric acid was used as a standard.

Protein determination: Protein was estimated by the method of Lowry et al. (7).

Triglyceride determination: Triglyceride was assayed by the method of Van Handel and Zilversmit. (8).

Carbohydrate determination: Carbohydrate was estimated by the phenol-sulfuric acid method (9).

Phospholipid determination: Phospholipid was estimated by the method of Bartlett (10).

Choresterol and choresterol ester determination: Choresterol and choresterol ester were determined by the method of Zak and Dickernman (11).

Table I Distribution of fat cell components during preparation of lipid micelles

Subfraction	Protein	α-Glycerophosphate dehydrogenase	Lipase	Esterase
	mg/g	μmole/g	μEq/g	μmole/g
Lipid micelles	2.1	4.5	4.4	35
Supernatant	7.1	19.6	1.7	16
Pellet	0.3	0.3	0	0

Table 2 Composition of lipid micelles

Fraction	Amount
	mg/g
Dry weight	159.0
Protein	2.1
Triglyceride	126.0
Sugar	0.5
Phospholipid	4.5
Choresterol ester	5.7
Choresterol	0.2

RESULTS AND DISCUSSION

Nuclei were seen in preparation of fat cells but not in preparation of lipid micelles, as shown in Fig. 1, indicating that most fat cells were disrupted during the preparation of the lipid micelles. Further evidence that the fat cells were disrupted was that most of the protein and α-glycerophosphate dehydrogenase which is a soluble enzyme, were found in

the supernatant fraction after disrupting the fat cells in hypotonic medium (Table I).

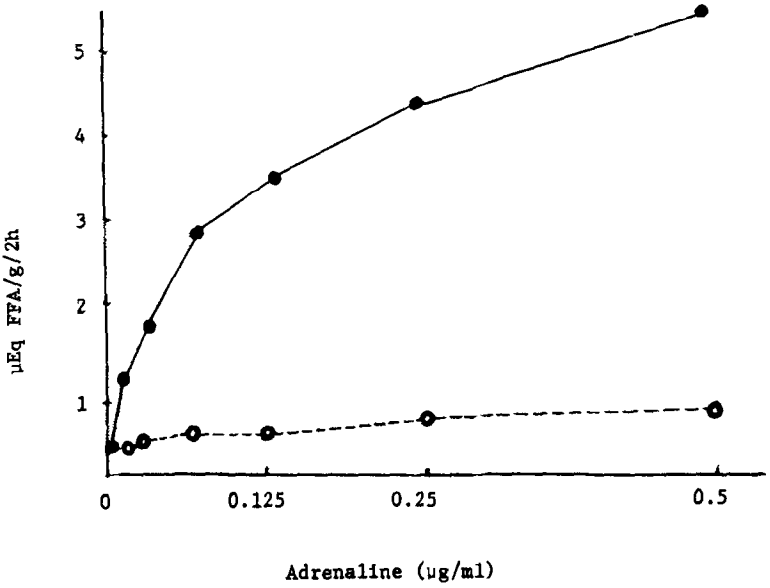

Fig. 2 Effect of adrenaline on lipolysis in lipid micelles
intact lipid micelles: ●——● Homogenized lipid micelles: o----o

In contrast with α-Glycerophosphate dehydrogenase, lipase and esterase were found to be associated with lipid micelles (Table I). The lipid micelles also contained protein, sugar, phospholipid, choresterol ester and choresterol, triglyceride beeing the main constituent, as shown in Table 2.

Volumes of 0.5 ml of lipid micell suspension, equivalent to 200 mg of the adipose tissue, were mixed with various amounts of adrenaline in 0.5 ml of Krebs Ringer phosphate buffer pH 7.4. The mixtures were incubated at 37° for 2 h and then Dole's extraction mixture was added and free fatty acids released were estimated by the method of Dole(6). Adrenaline stimulated release of free fatty acids considerably, as shown in Fig. 2. When the lipid micell suspension had been

homogenized by 10 strokes in a Teflon Homogenizer, no stimulation of lipolysis by adrenaline was observed (Fig. 2). These results suggest that some structure in the lipid micelles is required for the effect of adrenaline.

Adrenaline-induced lipolysis is widely thought to be due to stimulation of cyclic AMP which in turn activates hormone-sensitive lipase (2, 3, 12).

Recently, Huttunen et al. partially purified hormone-sensitive lipase from rat adipose tissue and demonstrated that its activation was effected via cyclic AMP-dependent protein kinase (4, 13).

Experiments are now in progress on whether this is also mechanism of adrenaline-induced lipolysis in lipid micelles.

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