ADIPOSE TISSUE: HORMONAL INFLUENCE ON DISTRIBUTION OF LIPOLYTIC ACTIVITY IN HOMOGENATE FRACTIONS

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The lipid-mobilizing action of hormones, such as epinephrine and ACTH is well established. Evidence suggests that this mobilizing action is due to stimulation of intracellular lipolysis, probably by activation of lipolytic enzyme systems within the cell (1, 2). Less well known is the mechanism by which the hormones exert their action and the locii of the enzyme(s) within the fat cell.

A lipolytic enzyme in cell-free extracts of the rat epididymal fat was stimulated by epinephrine (3) and the lipolytic activity of a solid fat cake, obtained from homogenates of rat epididymal fat pad, was enhanced 2-5 fold by epinephrine (4).

The experiments to be described were designed to locate the particular fraction (or fractions) of the disrupted tissue which responded to epinephrine and ACTH; these hormones were chosen since they appear to have similar actions on the release of free fatty acids (FFA) from adipose tissue, both in vivo and in vitro.

The distribution of lipolytic activity in adipose tissue was studied using paired rat epididymal fat pads; one pad served as a control, the contralateral pad was treated with the hormone.

METHODS

Epididymal fat pads were obtained from rats of the Sprague-Dawley strain, weighing between 250 and 275 grams, under Nembutal anesthesia.

After excision, fat pads were rinsed in ice-cold saline and each pad was homogenized for 1 minute in 5.0 ml of 0.06M phosphate buffer, pH 7.4. The hormone, where used, was added to the homogenizing medium, i.e., the tissue was homogenized in the presence of the hormone. The homogenates, centrifuged at 105,000g for 2 hours in a Model L Spinco, yielded four distinct fractions: (1) a top oil layer, (2) a solid fat cake, (3) an aqueous infranatant and (4) a sediment at the bottom of the tube.

Lipolytic activity of each fraction was measured by the release of FFA into an incubation medium composed of 0.06M phosphate buffer, pH 7.4 and bovine serum albumin; the latter served as an acceptor of the FFA released during lipolysis. The oil and fat cake fractions were dispersed in buffer by ultrasonic vibrations, these fractions required no additional substrate, albumin was added after emulsification. A buffer extract of the sedimented material was used for the lipolytic atudies of this fraction; Ediol (a coconut oil emulsion) served as substrate for the infranatant fractions and the extracts of the sediment. Samples for FFA determination were removed before and after 1 hour incubation at 37°C, lipolytic activity represents the difference between the two values.

Free fatty acids (FFA) were measured by a colorimetric method (5).

Protein nitrogen was measured by the method described by Lowry et al (6).

RESULTS AND DISCUSSION

Table 1 represents the lipolytic response in each fraction to epinephrine in terms of quantities of FFA on an absolute and percentage basis. Epinephrine exerted a profound stimulatory effect on the lipolytic activity of the oil layer as shown by a more than 1300% increase in enzymatic activity. A similar effect may also be seen when fat pads are incubated with epinephrine

TABLE 1. LIPOLYTIC RESPONSE OF ADIPOSE TISSUE HOMOGENATE TO EPINEPHRINE.

Fraction of Homogenate	Lipolytic activity μmoles FFA/mg protein N/hour		Percent Increase	Total activity μmoles FFA		Percent of Total activity	
	Control	Epinephrine (1 μg/ml)		Control	Epinephrine (1 μg/ml)	Control	Epinephrine (1 μg/ml)
Oil Layer	13.08 ± 3.26*	181.44 ± 32.07	1363	0.34	2.16	3	10
Fat Cake	9.91 ± 2.66	16.56 ± 1.07	67	4.94	9.29	37	46
Infranatant	7.78 ± 0.69	7.83 ± 0.27	0	5.65	5.68	42	27
Sediment	27.32 ± 4.02	33.64 ± 6.99	22	2.41	3.30	18	17

*Mean ± S.E. of Mean. Represents the average value of four experiments.

TABLE 2. LIPOLYTIC RESPONSE OF ADIPOSE TISSUE HOMOGENATE TO ACTH.

Fraction of Homogenate	Lipolytic activity μmoles FFA/mg protein N/hour		Percent Increase	Total activity μmoles FFA		Percent of Total activity	
	Control	ACTH (0.2 units/ml)		Control	ACTH (0.2 units/ml)	Control	ACTH (0.2 units/ml)
Oil Layer	13.04 ± 4.06*	0.00	0	0.40	0.00	4	0
Fat Cake	14.36 ± 3.81	33.68 ± 6.53	134	3.20	9.40	34	64
Infranatant	4.47 ± 0.60	4.14 ± 0.23	0	3.67	3.47	38	23
Sediment	18.81 ± 2.25	23.02 ± 3.21	22	2.09	1.77	24	13

*Mean ± S.E. of Mean. Represents the average value of four experiments.

before homogenization or when epinephrine is added to an incubation medium containing the oil fraction. Lipolytic activity of the fat cake was moderately stimulated by epinephrine and the infranatant fraction not at all. The increase in activity in the sediment was not statistically significant.

In the presence of epinephrine, the total activity of the oil and fat cake fractions was increased, 6.4 and 1.9 times, respectively. The total activity of the other two fractions did not differ markedly, with or without the hormone.

Table 2 describes the response to ACTH of the lipolytic activity of fractions from homogenized fat pads. ACTH abolished the lipolytic activity of the oil layer and enhanced that of the fat cake (134%). The activity of the infranatant fraction was unchanged by ACTH and the increased lipolysis by the sediment was of the same magnitude as seen with epinephrine.

By use of endogenous substrates it is possible to distinguish between lipolytic responses of adipose tissue to epinephrine and ACTH. The effect of these hormones on the oil and fat fractions suggest the possible presence of two separate enzyme systems, one of which is stimulated by epinephrine, the other by ACTH.

A comparison of the infranatant and sediment with the lipid fractions may not be valid because of substrate differences; however, they may be compared with each other; no difference was found in their response to the two hormones. It should be mentioned that the oil and fat fractions hydrolyze Ediol, furthermore, hydrolysis of Ediol is enhanced in the presence of epinephrine.

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