## BBA Report

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# Lipolytic activity of adipocyte ghosts

L. RONALD CRUM and DEANE N. CALVERT
*Department of Pharmacology, The Medical College of Wisconsin, Milwaukee, Wisc. 53233 (U.S.A.)*
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SUMMARY

Adipocyte ghosts made from isolated cells pretreated with $1 \cdot 10^{-6}$ M norepinephrine and $1 \cdot 10^{-3}$ M theophylline prior to hypotonic lysis showed significantly greater lipolytic activity (tributyrin hydrolysis) than control ghost preparations, provided that the ghosts were resuspended in a substrate-containing medium. When the ghosts were taken up in buffer alone and substrate was added during the assay step, no such stimulation of lipolytic activity was seen.

Recently several attempts have been made to isolate and characterize the "hormone-sensitive lipase" present in adipose tissue[1-3]. The results of these attempts have indicated that this enzyme (or enzymes) has a highly lipophilic nature and that it may be characterized as either a protein saturated with lipid substrate[4] or a lipoprotein[5]. These observations have suggested to us that the hormone-sensitive lipolytic activity of adipocytes may be due, at least in part, to a structural or membrane-bound enzyme. In support of such a possibility, we have found that catecholamine-stimulated lipolytic activity is associated with the membrane "ghosts" formed by the hypotonic lysis of isolated fat cells. Furthermore, the specific activity of this membrane-associated lipolytic activity is approximately 40 times greater than that reported by Allen *et al.*[6] for whole fat cells treated with a similar dose of hormone, and approximately 50 times greater than that found in broken cell preparations of isolated adipocytes (manuscript in preparation).

In this work isolated adipocytes were prepared from the epididymal fat pads of Holtzman rats (150–200 g) according to the method of Rodbell[7] as modified by Lech and Calvert[8]. Protein determinations were made using the method of Lowry[9] with bovine serum albumin as a standard. The isolated adipocytes thus prepared were divided into two equal portions, labelled A and B. The B cells were treated with $1 \cdot 10^{-6}$ M norepinephrine and $1 \cdot 10^{-3}$ M theophylline in Krebs–Ringer phosphate buffer; while the A cells, serving as a control, received Krebs–Ringer buffer alone. Following a 2-min exposure to the hormone

(or Krebs–Ringer buffer) adipocyte ghost preparations were made from both control and hormone-treated cells according to the method of Rodbell[10] with the following modifications: (1) the cells were lysed *via* exposure to glass-distilled water and agitation for a period of 5 min; (2) these broken-cell preparations (or homogenates, as they are referred to in Table I) were centrifuged at a force of 100 000 × *g* for a period of 15 min; and (3) the pellet thus formed was separated from the fat layer and infranatant fluid and was resuspended *via* sonication of 1.67 sec/ml (Branson Sonifier Cell Disruptor, microtip setting of 7) in either Krebs–Ringer buffer alone or 0.1 M tributyrin emulsion in Krebs–Ringer phosphate buffer.

The assay system for lipolytic activity used in these experiments consisted of 0.2 ml of the resuspended ghosts in the following medium: (1) 0.02 mole phosphate buffer, pH 7.0; (2) 4% bovine serum albumin; and (3) glass-distilled water up to a 1.0 ml assay volume incubated for 1 h at 37°. In those experiments in which the ghosts were resuspended in Krebs–Ringer buffer alone, 0.02 mole of tributyrin emulsion was also added to the assay medium to serve as substrate. The butyric acid liberated during the incubation period was then extracted and titrated according to the method of Dole and Meinertz[11]. Corrections were made for the partition of butyrate between heptane and water. In addition, the lipolytic activities of the original broken cell preparations were also assayed in order to insure that the hormone-treated (B) adipocytes were activated prior to any experimental manipulations.

Table I shows the results of resuspending both control (A) and hormone-treated (B) ghost pellets in tributyrin in Krebs–Ringer buffer. These results are expressed as the change in free fatty acid (or butyrate) (60 min titration *minus* zero time titration) per mg protein in the assay per h. It can be seen in Table I that the lipolytic activity of the hormone-treated (B) homogenates was significantly greater ($P < 0.01$) than that of the control (A) homogenates prior to the ultracentrifugation of both preparations. After ultracentrifugation and separation of the centrifugates into "fat + infranatant" and pellet fractions, the lipolytic activities, both control and hormone-stimulated, of the original broken-cell preparations were found to be associated primarily with the pellet fractions. The "fat + infranatant" preparations from both control (A) and hormone-treated (B) cells showed little lipolytic activity and there was no significant difference between the activities of the A and B preparations. On the other hand, the pellets (resuspended in tributyrin in Krebs–Ringer buffer) showed greatly stimulated lipolytic activity; and in addition, the difference between hormone stimulated (B) and control (A) preparations was retained ($P = 0.05$).

Fig.1 shows the time course of the hydrolysis of tributyrin by both catecholamine-stimulated (broken line) and control (solid line) ghost pellets resuspended in tributyrin in Krebs–Ringer phosphate buffer. It can be seen that both preparations produce a linear liberation of butyrate over the 1-h incubation period. In addition, Fig.1 shows that the hormone-stimulated (B) ghosts liberated approximately 2 times more butyrate over the 1-h period than did the control (A) ghosts.

When the ghost pellets were taken up in Krebs–Ringer phosphate buffer alone, however, and the tributyrin substrate was added to the assay medium, the hormone-stimulated lipolytic activity of the ghost preparations was lost. The results of this procedure are presented in Fig.2, where it can be seen again that when the ghost pellets were taken up

TABLE I

LIPOLYTIC ACTIVITIES OF ORIGINAL HOMOGENATES, "FAT + INFRANATANT" AND GHOST PELLET FRACTIONS

| *Preparation* | *Substrate* | *Total activity*** *(μequiv free fatty acids/h per total volume)* | *Total protein content (mg)* | *Specific activity*** *(μequiv free fatty acids/mg protein per h)* |
|---|---|---|---|---|
| Homogenate | | | | |
| A | Endogenous | 2.75 ± 0.04 | 8.09 | 0.34 ± 0.12 |
| B | Endogenous | 16.50 ± 0.10 | 8.29 | 1.99 ± 0.28 |
| Fat + infranatant* | | | | |
| A | Endogenous | 1.25 ± 0.02 | 7.5 | 0.17 ± 0.06 |
| B | Endogenous | 1.50 ± 0.03 | 7.5 | 0.20 ± 0.10 |
| Ghosts (pellet)* | | | | |
| A | Exogenous tributyrin | 63.30 ± 0.50 | 0.38 | 164.65 ± 18.03 |
| B | Exogenous tributyrin | 89.40 ± 1.40 | 0.39 | 231.35 ± 28.87 |

*After centrifugation at 100 000 x g for 15 min.
**Values represent means of five experiments ± S.E.

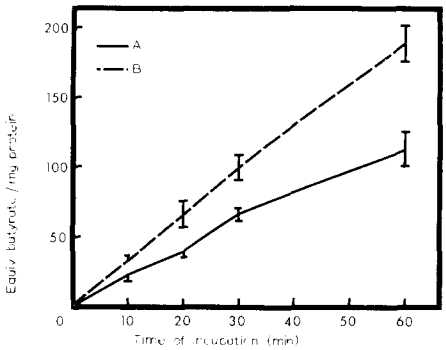


Fig. 1. Time course of butyrate release by control (A) and hormone-treated (B) ghost preparations resuspended in tributyrin Krebs–Ringer phosphate. Values represent means of 5 experiments ± S.E. Incubation temperature was 37°. A, control; B, treated with $1 \cdot 10^{-6}$ M norepinephrine *plus* $1 \cdot 10^{-3}$ M theophylline.

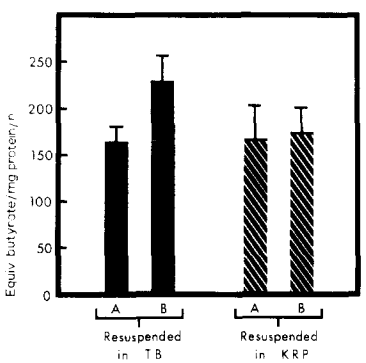


Fig. 2. Tributyrin hydrolysis by adipocyte ghosts. TB refers to tributyrin emulsion in Krebs–Ringer phosphate (KRP) buffer. A represents ghosts made from control adipocytes; B represents ghosts made from cells pretreated with $1 \cdot 10^{-6}$ M norepinephrine and $1 \cdot 10^{-3}$ M theophylline. Values represent means of 5 experiments ± S.E. Incubation period was 60 min at 37°.

| *Analysis of variance* | *d.f.* | *F* | *Significance level* |
|---|---|---|---|
| $A_{TB}$ *vs.* $B_{TB}$ | 1 and 10 | 7.23 | 0.05 |
| $A_{KRP}$ *vs.* $B_{KRP}$ | 1 and 8 | 0.04 | not significant |

in tributyrin in Krebs–Ringer buffer the lipolytic activity of the hormone-treated (B) ghosts was greater than that of the control (A) ghosts. When, however, the ghosts were resuspended in Krebs–Ringer buffer alone the activity of the hormone-treated (B) ghosts against tributyrin in the assay medium was approximately equal to that of the control (A) preparations.

A possible explanation for these observations is that the "hormone-sensitive lipase" is bound or somehow associated with the inner surface of the plasma (or other) membrane

of the adipose cell. Hypotonic lysis of the fat cells ruptures the cell membranes thus allowing the cytoplasm and natural triglyceride substrate (oil droplet) to escape into the surrounding medium. The broken membranes thus formed apparently close around the hypotonic medium forming "adipocyte ghosts" and the centrifugation step precipitates the ghost (*plus* some mitochondria and microsomes)[10]. One of two possible factors may account for the incorporation of tributyrin upon resuspension of the ghosts: (1) the force of the ultracentrifugation may compress the ghosts, possibly forcing the hypotonic medium from their interiors and permitting them to enclose a portion of the medium in which they are resuspended; or (2) the sonication during the resuspension of the ghosts may rupture the membranes allowing substrate-containing medium to enter. Subsequently, when the ghosts are resuspended in tributyrin in Krebs–Ringer buffer the lipase (on the inner surface of the ghost membrane?) is able to catalyze the hydrolysis of the enclosed tributyrin substrate. When, however, the ghosts are resuspended in Krebs–Ringer buffer alone, no tributyrin enters the newly formed ghosts, the tributyrin emulsion droplets being too large to enter the ghosts once the spheres have reformed. Consequently, no hormone-stimulated lipolytic activity is seen in this preparation. The control (A) activity is probably due to the presence of one or more enzymes which possess tributyrinase activity but which are not hormone sensitive. Preliminary microscopic examinations of adipocytes and adipocyte ghosts taken up in tributyrin or Krebs–Ringer buffer are consistent with this view. It should be noted, however, that definitive evidence to localize the lipase specifically on the inner surface of the adipocyte plasma membrane is not contained in this report; enzymatic tests or electron microscopic examination to evaluate the extent of mitochondrial and microsomal contamination have not yet been undertaken.

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