Release of Lipoprotein Lipase Activity from Isolated Fat Cells

II. EFFECT OF HEPARIN*

(Received for publication, April 2, 1973)

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SUMMARY

Addition of heparin to isolated fat cells incubated at 23° resulted in a 3-fold increase in release of lipoprotein lipase activity into the incubation medium. This increase in medium lipoprotein lipase activity was not due to enhancement or stabilization of lipase activity present in the medium. The maximum increase in lipoprotein lipase activity was obtained on addition of 0.1 unit of heparin per ml to the cells. The heparin-stimulated release of lipoprotein lipase was almost completely inhibited by the metabolic inhibitors, sodium cyanide and Antimycin-A. When protein synthesis was blocked with cycloheximide, and heparin added to the incubation medium, neither the release of lipoprotein lipase activity nor the intracellular enzyme activity was affected for 120 min. At the end of this 120-min incubation there was approximately 5 times as much lipoprotein lipase activity in the medium as in the cells initially. These studies suggest that intracellular lipoprotein lipase undergoes activation in association with heparin-stimulated release from fat cells.

Heparin injection is known to cause a loss of turbidity in the plasma from postabsorptive subjects. This so-called clearing of plasma has been shown to be due to the action on plasma triglyceride (1) of lipolytic enzymes, which are released from tissues by heparin injection. This circulating lipolytic activity has been shown to have properties similar to those of the lipoprotein lipase (glycerol ester hydrolase, EC 3.1.1.3) found in adipose tissue and heart muscle (2). It has been suggested that this enzyme regulates the entry of plasma triglyceride fatty acid into tissues (3).

Although it is known that heparin releases lipoprotein lipase

* This work is supported in part by United States Public Health Service Grant 4706 from the National Institute of Arthritis and Metabolic Diseases and by a Grant-in-Aid from American Heart Association with funds contributed in part by the Los Angeles County Heart Association.

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immediate doubling of the intracellular lipoprotein lipase activity. The fat cells were incubated in the presence and absence of heparin, 10 units per ml for 45 min. Lipoprotein lipase activity was assayed in the medium and infranatant fractions of homogenates of these cells. The values are the means of duplicate analysis. The data are expressed FFA per ml per hour per g of fat cells.

separated from fat cells by centrifugation (60 × g) for 30 s and stored in an ice bath (1 to 2 hours) until assayed for lipoprotein lipase activity. The fat cells were washed, resuspended in 2 ml of the incubation buffer described below, and quickly homogenized in 5-ml Ten-Broeck homogenizers in an ice bath. Cell homogenates were centrifuged at 800 × g for 15 min at 4°. The infranatant fraction was separated from the fat cells and also stored in an ice bath until assayed. The infranatant fraction of homogenates was designated intracellular lipoprotein lipase activity. The incubation medium was composed of Krebs-Ringer bicarbonate buffer (pH 7.4) containing glucose, 1 mg per ml, and Fraction V bovine albumin, 3 mg per ml. Lipoprotein lipase was assayed by a radioactive assay utilizing glyceryl tri[l-14C]oleate as a substrate (9). Fat cell dry weight was determined after 48-hour desiccation on Millipore filters (9). Heparin (140 units per mg) and Antimycin-A were purchased from Celhiochem, La Jolla, Calif. Lipoprotein lipase activity is expressed as microequivalents of FFA1 per ml of incubation medium per hour or microequivalents of FFA per ml of infranatant fraction per hour.

RESULTS

When isolated fat cells were incubated in the absence of heparin, the increase of lipoprotein lipase activity in the medium was linear with time (Fig. 1). In the presence of heparin, the increase in medium lipoprotein lipase activity was also linear (Fig. 1). However, with heparin the rate of appearance of lipase activity was considerably greater, so that after 45 min of incubation, approximately 2.5 times as much activity was present. Following the addition of heparin to fat cells, there is an immediate doubling of the intracellular lipoprotein lipase activity and this activity remains essentially constant during the incubation period. The level of intracellular lipoprotein lipase activity of cells incubated in the absence of heparin is also constant, but is at a lower level than heparin-treated cells (Fig. 1).

Previously, Pokrajac et al. (6) suggested that the increase in lipoprotein lipase activity in the incubation medium following addition of heparin to isolated fat cells was due to stabilization or activation of lipoprotein lipase activity in the medium. Our study shows that when cell-free medium containing previously released lipoprotein lipase activity was incubated for 30 min in the absence of heparin, little or no change in enzyme activity was observed. The addition of heparin did not significantly alter this activity found in the medium (Fig. 2). It would, therefore, appear that the increase in lipoprotein lipase activity in the medium shown in Fig. 1 in the presence of heparin was due neither to activation nor to stabilization of lipase activity present in cell-free medium, but rather to increased release of activity from the cells.

Sodium chloride inhibition and activation by serum are two common criteria used to characterize lipoprotein lipase activity. When serum was removed from the lipase assay, a very large decrease (75%) of lipoprotein lipase activity was observed in the medium from fat cells whether incubated in the presence or absence of heparin (Experiments 1 and 3, Table I). In contrast, NaCl inhibition of lipoprotein lipase activity differed in the presence or absence of heparin. In the medium obtained from fat cells incubated in the presence of heparin only a 30% loss of lipase activity was observed (Experiment 2, Table I). However, a 90% inhibition of lipoprotein lipase activity by 1 m NaCl was found in the medium obtained from fat cells incubated without heparin (Experiment 4, Table I).

![Fig. 1. The effect of heparin on appearance of lipoprotein lipase activity in the incubation medium. Fat cells were incubated in the presence and absence of heparin, 10 units per ml for 45 min. Lipoprotein lipase activity was assayed in the medium and infranatant fractions of homogenates of these cells. The values are the means of duplicate analysis. The data are expressed FFA per ml per hour per g of fat cells.

![Fig. 2. Stability of lipoprotein lipase activity. Fat cells were prepared and incubated for 45 min. The medium was separated from fat cells and was incubated at 23° in the presence and absence of heparin, 10 units per ml. Following incubation, this cell-free medium was assayed for lipoprotein lipase activity. Results are the means of duplicate analysis.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Heparin in incubation</th>
<th>Serum in assay</th>
<th>Preincubation NaCl</th>
<th>Lipoprotein lipase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>0.085 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.022 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>0.066 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.046 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.032 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.009 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.003 ± 0.003</td>
</tr>
</tbody>
</table>

1 The abbreviation used is: FFA, free fatty acid.
It seemed quite possible that these differences in sensitivity of lipoprotein lipase activity to NaCl inhibition result from the release of a different form of lipoprotein lipase on addition of heparin to fat cells. Alternatively, the interaction of heparin with lipoprotein lipase could ‘protect’ the enzyme from inactivation by NaCl (10). This latter possibility was tested by separating incubation medium containing lipoprotein lipase activity released from fat cells in the absence of heparin. Heparin was added to this cell-free medium and incubated in the presence or absence of NaCl prior to assay for lipoprotein lipase activity. As shown in Table II, the addition of heparin to cell-free medium containing lipoprotein lipase activity produced approximately the same protection from inhibition by 1 M NaCl as found in Table I, Experiment 2, where heparin was utilized to release lipoprotein lipase activity from fat cells. Although these data do not rule out the possibility that different forms of lipoprotein lipase are released from fat cells in the presence or absence of heparin, they suggest that a molecular interaction of heparin with the enzyme, or substrate, or both, in the assay system is most likely.

The effect of heparin concentration on release of lipoprotein lipase activity shows that maximum enzyme release was obtained at 0.1 unit per ml of heparin. No further activity was released even when the concentration of heparin was increased 100-fold (Fig. 3).

### Table II

**Effect of NaCl on non-heparin-released lipoprotein lipase activity in presence of added heparin**

<table>
<thead>
<tr>
<th>NaCl</th>
<th>Lipoprotein lipase activity (μg FFA/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M NaCl</td>
<td>0.113 ± 0.002</td>
</tr>
<tr>
<td>+</td>
<td>0.074 ± 0.003</td>
</tr>
</tbody>
</table>

Fig. 3. Effect of heparin concentration on release of lipoprotein lipase activity. Fat cells were prepared and incubated with and without various concentrations of heparin. Following 15 min of incubation, the medium was separated from the fat cells and assayed for lipoprotein lipase activity. The values obtained in the absence of heparin was plotted as 0.001 unit on the logarithmic abscissa. Results are the mean of duplicate analysis. The data are expressed as FFA per ml per hour per g of fat cells.

![Graph showing the effect of heparin concentration on lipoprotein lipase activity](http://www.jbc.org/)

It has been proposed that the release of lipoprotein lipase activity from adipose tissue by heparin is due to alteration of electrostatic forces binding the enzyme (4). If this be true, heparin release of lipoprotein lipase activity from isolated fat cells might also be due to alterations of binding electrostatic forces, so that enzyme release would not require metabolic energy. To determine whether metabolic energy is required for release, or activation, or both, of lipoprotein lipase activity following the addition of heparin to isolated fat cells, sodium cyanide or Antimycin-A was added to incubating fat cells and release of enzyme activity was measured. About 10 min following addition of NaCN (Fig. 4A) or Antimycin-A (Fig. 4B) to fat cells, the amount of lipoprotein lipase activity released in the medium was drastically reduced.

We have shown that for isolated fat cells the intracellular level and rate of release of lipoprotein lipase activity in the absence of heparin are not affected by cycloheximide during 45 min of incubation (9). Fig. 5 shows similar findings in the presence of heparin. When fat cells were incubated 120 min in the absence of cycloheximide, the rate of release was approximately linear for 60 min and then decreased somewhat during the next 60 min. During the same time period, the intracellular levels of lipoprotein lipase activity remained essentially unchanged.

**DISCUSSION**

Several studies have shown release of lipoprotein lipase activity from isolated cells following the addition of heparin (6, 11, 12). Pokrajac et al. (6) found no change in intracellular lipoprotein lipase activity in response to heparin, while Patten and Hollenburg (12) reported a decrease in cellular lipoprotein lipase activity corresponding to the amount of enzyme activity that was released into the medium. In the present experiments the intracellular level of lipoprotein lipase activity remained relatively constant during incubation. Therefore, our findings are in agreement with the results reported by Pokrajac et al. (6).

The effect of heparin on lipoprotein lipase activity released by isolated fat cells into the incubation medium differs greatly from its effect on intracellular lipoprotein lipase activity. While
heparin has no effect on extracellular lipoprotein lipase (Fig. 2) when added to homogenates of fat cells, with or without incubation, doubles lipoprotein lipase activity (Fig. 1). The reason for these differences in activity on addition of heparin is not clear. It is of interest that Garfinkel and Schotz (13) have separated from rat adipose tissue two species of lipoprotein lipase activity differing in their response to heparin.

We have shown that in the absence of heparin release and intracellular levels of lipoprotein lipase activity were not affected by cycloheximide and have proposed that activation of intracellular lipoprotein lipase accompanied release of lipoprotein lipase activity into the medium (9). In Fig. 5, we show a similar lack of effect of cycloheximide on fat cells incubated in the presence of heparin. As we previously observed (9), a much greater amount of lipoprotein lipase activity is released than can be accounted for by the amount of lipoprotein lipase activity in the cells, unless the rate of lipoprotein lipase synthesis is equal to that of lipoprotein lipase activity release. Only if these rates were equal, would a constant intracellular concentration of lipoprotein lipase be maintained. However, when protein synthesis was blocked with cycloheximide, the level of lipoprotein lipase activity in homogenates of isolated fat cells, as well as its rate of release, remained unchanged. Therefore, in the presence or absence of heparin lipoprotein lipase activity appeared to undergo activation in association with its release from fat cells.

Since it has been proposed that heparin causes release of lipoprotein lipase from adipose tissue by altering electrostatic binding forces (4), it would seem quite possible that release of lipoprotein lipase activity from fat cells in the presence or absence of heparin could be due to different mechanisms. However, our findings indicate that release of lipoprotein lipase activity from fat cells in the presence or absence of heparin is similar in that the release, or activation, or both, requires metabolic energy but not protein synthesis. It, therefore, appears that the mechanism of this release is different from that for release of lipoprotein lipase activity by heparin from adipose tissue which has been reported not to require metabolic energy (4).

Acknowledgment—We wish to thank Judith Nikazy for her most skilled technical assistance.

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