Metabolism of Isolated Fat Cells

III. THE SIMILAR INHIBITORY ACTION OF PHOSPHOLIPASE C (CLOSTRIDIUM PERFRINGENS α TOXIN) AND OF INSULIN ON LIPOLYSIS STIMULATED BY LIPOLYTIC HORMONES AND THEOPHYLLINE

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SUMMARY

A study was made of the effects of phospholipase C (Clostridium perfringens α toxin) and of insulin on lipolysis by fat cells isolated from epididymal adipose tissue of the rat. Both insulin and phospholipase C inhibited, in the absence of glucose in the incubation medium, the release of fatty acids and glycerol that was stimulated by submaximal concentrations of adrenocorticotropic hormone, epinephrine, glucagon, and theophylline. Both substances failed to inhibit the lipolytic action of high concentrations of adrenocorticotropic hormone and theophylline.

The similar inhibitory action of phospholipase C and of insulin on lipolysis, an action that is independent of their stimulatory effects on glucose transport, provides additional evidence that both may be acting on a common parameter, possibly the plasma membrane, of the fat cell.

EXPERIMENTAL PROCEDURE

Male Sprague-Dawley rats (190 to 220 g), fed a Purina laboratory chow, were used in these studies. The procedures used for isolating, dispensing, and incubating fat cells from rat adipose tissue have been described elsewhere (7). Glucose was omitted from the medium during treatment of adipose tissue with collagenase for 14 hours and during the subsequent washing of the cells. Fat cells were incubated in a 4% solution of albumin (bovine Fraction V, Armour's Lot 22312) in bicarbonate buffer (7) that was adjusted to pH 7.4 in an atmosphere of 95% O₂-5% CO₂. The albumin medium was prepared fresh for each experiment and was filtered through a 0.22-μ Millipore filter.

The isolated fat cells were preincubated for 15 min at 37°, with shaking, in 1.75 ml of albumin-bicarbonate medium that contained egg lipoprotein, 2.5 pmoles of phospholipid per ml, prepared as described previously (6). When added, phospholipase C and insulin were present during the preincubation period. α antitoxin (Lilly's equine C. perfringens, 400 units per ml), which stops the action of phospholipase C (6), was then added to all flasks to give a final concentration of 1 unit per ml. After the addition of the lipolytic agent to appropriate flasks, cells were incubated for 1 hour at 37° in a final volume of 2 ml of albumin-bicarbonate medium. The gas phase was 95% O₂-5% CO₂.

The content of glycerol in the medium was determined, without deproteinization, by a modification of the method of Wieland (8). Fatty acid (unesterified) content of the medium was analyzed by the procedure of Dole and Meinertz (9). The method of measuring the oxidation of glucose-1-¹⁴C to ¹⁴CO₂ by fat cells has been presented elsewhere (6, 7).

Insulin inhibits the stimulatory effects of several hormones on lipolysis by intact adipose tissue (1-3) and by isolated fat cells (4). It also reduces basal lipolysis observed in intact adipose tissue obtained from fasting-refed (5) or in isolated fat cells from fasting rats (4). This antilipolytic action of insulin is independent of its effects on glucose transport or metabolism.

It was shown in a previous report (6) that phospholipase C (Clostridium perfringens α toxin) mimics the effects of insulin on glucose metabolism and protein synthesis by fat cells isolated from rat adipose tissue. This report describes the effects of phospholipase C on lipolysis by isolated fat cells that is stimulated by epinephrine, glucagon, adrenocorticotropic hormone, and theophylline. The results indicate that, like insulin, phospholipase C treatment of fat cells also results in the inhibition of lipolysis by a mechanism that is independent of its action on glucose utilization.

RESULTS

It was shown previously that phospholipase C, at relatively high concentrations, causes lysis of the fat cell and a concomitant loss of glucose oxidation in response to insulin (6). Insulin inhibits the stimulatory effects of several hormones on lipolysis by intact adipose tissue (1-3) and by isolated fat cells (4). It also reduces basal lipolysis observed in intact adipose tissue obtained from fasting-refed (5) or in isolated fat cells from fasting rats (4). This antilipolytic action of insulin is independent of its effects on glucose transport or metabolism.

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ent study, lysis of the fat cells with phospholipase C also resulted in the complete loss of the effects of agents that stimulate lipolysis in the fat cell. Since it was desired to examine the effects of phospholipase C on lipolysis by intact cells, egg lipoprotein, which diminishes the lytic effect of the enzyme (6), was added to the medium during preincubation of the cells with enzyme. The extent of cell lysis was determined in each experiment by comparing the amount of glucose oxidized (glucose-1-14C to 14CO2), in the presence of insulin, by an equivalent amount of untreated and phospholipase C-treated cells. Loss of insulin response is equivalent to cell lysis (6). Experiments were discarded if glucose oxidation, in response to insulin, by phospholipase C-treated cells was less than 90% of the insulin response of untreated cells.

In the absence of lipolytic hormones (ACTH, epinephrine, glucagon) or of theophylline, fat cells did not release fatty acids during 1 hour of incubation. The absence of basal lipolysis (glycerol or fatty acid release) by isolated fat cells during a 1-hour incubation has been reported previously (10). As shown in Table I, theophylline (0.1 mM) stimulated the release of fatty acids. Others have observed this same effect of theophylline on fat cells (4, 11, 12). Phospholipase C, at 1 µg per ml of preincubation medium, had no effect on the stimulatory action of theophylline. However, at 2.5 µg per ml, phospholipase C caused a 50% reduction in fatty acid release; 77% reduction was obtained with 6.2 µg of enzyme per ml.

Addition of C. perfringens α antitoxin at zero time rather than after the 15-min preincubation period (see "Experimental Procedure") prevented the effects of phospholipase both on the inhibition of fatty acid release and on stimulation of basal glucose oxidation (Table I). The latter effect was obtained at the same concentrations of the enzyme that caused the reduction of fatty acid release in response to theophylline. Neither effect on the cells was observed if phospholipase C and lipoprotein were incubated for 15 min before the addition of antitoxin and fat cells.

Table I

Effects of phospholipase C on fatty acid release in response to theophylline and on glucose-1-14C oxidation in presence and absence of insulin

An average of 35 mg of fat cells was preincubated for 15 min at 37° in 1.75 ml of albumin-bicarbonate buffer containing 2.5 µmoles of lipoprotein phospholipid per ml and the indicated concentrations of phospholipase C. Subsequently, 1 unit of antitoxin was added. Fatty acid release was measured after 1 hour of incubation in 2 ml of medium. Glucose-1-14C oxidation by the same batch of cells was measured after 2 hours of incubation in 2 ml of albumin-bicarbonate medium that contained 3 mM glucose-1-14C and, when added, 1 milliumlit of insulin per ml. Values are the mean (± s.e.) of 3 experiments.

| Phospholipase C added to preincubation medium | Antitoxin added to preincubation medium (1 milliumlit/ml) | Free fatty acid released with theophylline (0.1 µm) | Glucose-1-14C Oxidized insulin + insulin µg/ml | µg X 1 hr | µg X 2 hrs | 18.3 ± 0.2

<table>
<thead>
<tr>
<th>µg/ml</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>159 ± 40</td>
<td>1.8 ± 0.3</td>
<td>8.6 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>-</td>
<td>147 ± 30</td>
<td>1.7 ± 0.4</td>
<td>8.5 ± 0.7</td>
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<td></td>
</tr>
<tr>
<td>2.5</td>
<td>-</td>
<td>77 ± 5*</td>
<td>3.4 ± 0.4*</td>
<td>8.4 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.2</td>
<td>-</td>
<td>37 ± 8*</td>
<td>4.7 ± 0.3*</td>
<td>7.6 ± 0.6</td>
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<td></td>
</tr>
<tr>
<td>6.2</td>
<td>+</td>
<td>163 ± 25</td>
<td>1.8 ± 0.3</td>
<td>8.6 ± 0.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant effect of phospholipase C (p < 0.05).

Table II

Effects of insulin and phospholipase C on fatty acid release by fat cells treated with submaximal and maximal concentrations of theophylline

An average of 28 mg of fat cells was incubated under the conditions described in Table I. Values are the mean (± s.e.) of two experiments.

<table>
<thead>
<tr>
<th>Theophylline concentration</th>
<th>Free fatty acid released in response to theophylline</th>
<th>µg/m X 1 hr</th>
<th>µg/m X 2 hrs</th>
<th>µg/m X 3 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Insulin (200 micromoles per ml)</td>
<td>Phospholipase C (2.5 µg per ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>121 ± 10</td>
<td>24 ± 3*</td>
<td>5 ± 0.5*</td>
<td>79 ± 5*</td>
</tr>
<tr>
<td>1.0</td>
<td>176 ± 12</td>
<td>150 ± 9</td>
<td>130 ± 13</td>
<td>177 ± 9</td>
</tr>
</tbody>
</table>

* Significant effect of either insulin or phospholipase C (p < 0.05).

Table III

Effect of insulin and phospholipase C on fatty acid release in response to submaximal and maximal concentrations of ACTH

An average of 33 mg of fat cells per flask was incubated under the conditions described in Table I. Values are the mean (± s.e.) of 4 experiments.

<table>
<thead>
<tr>
<th>ACTH added</th>
<th>Free fatty acid released in response to ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/m</td>
<td>µg/m X 1 hr</td>
</tr>
<tr>
<td>0.01</td>
<td>90 ± 20</td>
</tr>
<tr>
<td>1.00</td>
<td>156 ± 25</td>
</tr>
</tbody>
</table>

* Significant effect of insulin or phospholipase C (p < 0.01).

Fig. 1. Inhibition by phospholipase C and insulin of glycerol release (lipolysis) stimulated by lipolytic hormones and by theophylline. Incubation conditions are the same as described in Table I. An average of 21 mg of fat cells was incubated per flask. Vertical bars are the means of 4 experiments. Standard error of the mean is indicated by the length of the vertical lines.

In the experiments described in Table I, phospholipase C had no effect on glucose oxidation in response to insulin, which indicates that its inhibitory action was not due to lysis of the fat cells.

Experiments comparing the effects of insulin and phospholipase C on fatty acid release in response to theophylline are described in Table II. Insulin, at 10 micromoles per ml, reduced
fatty acid release by 80%; at 1000 microunits per ml, insulin caused an almost complete reduction of fatty acid release in response to 0.1 mM theophylline. Phospholipase C, 2.5 and 6.2 μg per ml, was not as effective as insulin in reducing the stimulatory action of 0.1 mM theophylline.

Insulin and phospholipase C did not block, even at their higher concentrations, fatty acid release stimulated by 1 mM theophylline (Table II). The same results were obtained when ACTH was used as the lipolytic stimulant, as shown in Table III. Insulin and phospholipase C were equally effective in reducing the release of fatty acids in response to 0.01 μg of ACTH per ml. However, they failed to block the effects of 1 μg of ACTH per ml. The slight decrease in fatty acid release with the higher concentrations of ACTH and phospholipase C may have been caused by lysis of a small percentage (10% or less) of the cells by the enzyme.

Glycerol release from fat cells is a measure of the complete hydrolysis of triglycerides in fat cells (16). As shown in Fig. 1, suboptimal concentrations of ACTH (0.01 μg per ml), epinephrine (0.1 μg per ml), glucagon (0.15 μg per ml), and theophylline (0.1 mM) stimulated the release of glycerol. Insulin and phospholipase C reduced the stimulatory effect of all four lipolytic agents. As was shown in Table II for fatty acid release, the effect of 1 mM theophylline on glycerol release was not inhibited by phospholipase C or insulin.

**DISCUSSION**

The present findings indicate that phospholipase C mimics the antilipolytic effect of insulin on fat cells. The antilipolytic effects of both insulin and phospholipase C were obtained in the absence of glucose in the medium, which indicates that their effects on lipolysis are separate from their effects on glucose transport. The finding that the antilipolytic action of phospholipase C was only manifested at concentrations that also resulted in an increase in glucose utilization suggests that the same degree of phospholipid hydrolysis by the enzyme is necessary for these effects. Insulin inhibits lipolysis at concentrations as low as 1 microunit per ml, which is the lowest limit at which its effect on glucose metabolism can be detected with isolated fat cells.

It has been suggested that the lipolytic process in fat cells is dependent on the concentration of 3',5'-cyclic-AMP (cyclic-AMP), a nucleotide that activates a triglyceride lipase in homogenates of adipose tissue (17). The steady state concentration of cyclic-AMP depends on the balance between its formation which is catalyzed by adenyl cyclase (15) and on its breakdown which is catalyzed by a specific phosphodiesterase (16). Lipo-

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