Response of Trypsin-treated Brown and White Fat Cells to Hormones

PREFERENTIAL INHIBITION OF INSULIN ACTION*

(Received for publication, March 31, 1969)

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SUMMARY

Insulin did not stimulate glucose metabolism or inhibit lipolysis in brown fat cells isolated by digestion of rat interscapular brown adipose tissue with collagenase plus 1 mg of trypsin per ml. Trypsin-treated cells responded normally to catecholamines and to the antilipolytic agent, prostaglandin E\textsubscript{1}. The stimulation of respiration by lipolytic agents and free fatty acids was not affected by trypsin digestion, but the potentioniation of octanoate-induced respiration by insulin was abolished.

Incubation of white adipose tissue from rats with trypsin plus collagenase abolished the ability of insulin to inhibit lipolysis and stimulate glucose metabolism but did not affect the antilipolytic action of prostaglandin E\textsubscript{1}. Trypsin-treated white fat cells metabolized less glucose than cells isolated with collagenase alone but had a normal response to lipolytic agents such as theophylline, growth hormone plus dexamethasone, and catecholamines. The failure of trypsin-treated cells to respond to insulin was not due to destruction of insulin, because medium containing insulin which had been incubated with trypsin-treated fat cells stimulated the metabolism of glucose by cells isolated with collagenase only. Trypsin treatment abolished the stimulation of white fat cell glucose metabolism seen in the presence of "bound insulin" but not that due to oxytocin. These studies indicate that trypsin treatment selectively inactivates the response of fat cells to insulin and "bound insulin," but not to oxytocin, prostaglandin E\textsubscript{1}, or lipolytic agents.

Kono has reported in a preliminary communication (1) that incubation of white adipose tissue for 1 hour with large amounts (1 mg per ml) of proteolytic enzymes such as trypsin resulted in failure of the tissue to respond to insulin during a subsequent incubation. Kono also found that trypsin treatment did not alter the lipolytic response of the tissue to adrenocorticotrophic hormone or epinephrine (1).

The present studies were designed to investigate the effect of digests both brown and white adipose tissue with trypsin plus collagenase on the subsequent response of the brown and white fat cells to a variety of agents. The present studies indicate that trypsin digestion selectively reduces the sensitivity of fat cells to insulin and "bound insulin."

EXPERIMENTAL PROCEDURE

White fat cells were obtained by enzymatic digestion of the parametrial adipose tissue and brown fat cells by digestion of the dorsal interscapular brown adipose tissue of normal female rats (Charles River CD strain). The rats (130 to 150 G) were maintained on laboratory chow for 7 to 14 days after shipment. White fat cells were isolated by a modification of the procedure of Rodbell (2). In each experiment the parametrial adipose tissue from 3 or more rats was removed and placed in a Petri dish with 10 ml of 1% albumin buffer. The cut pieces of adipose tissue were blotted and divided between two 1-oz plastic bottles, one containing trypsin and one without. Each bottle contained 3 to 5 ml of 4% albumin in phosphate buffer and 0.5 mg of crude collagenase per ml (Clostridium histolyticum, 150 units per mg, from Worthington) without added glucose. The phosphate buffer contained: NaCl, 128 \textmu M; KCl, 5.2 \textmu M; CaCl\textsubscript{2}, 1.4 \textmu M; MgSO\textsubscript{4}, 1.4 \textmu M; KCl, 5.2 \textmu M; and Na\textsubscript{2}HPO\textsubscript{4}, 10 \textmu M. The albumin buffer was made up fresh daily and the pH was adjusted to 7.4 with sodium hydroxide, after addition of 4% bovine Fraction V powder.

The albumin preparations referred to as A-27 and A-709 albumin were Lots D-27008 and E-30709, respectively, which were obtained from Armour. P-55 albumin refers to Lot P-55 obtained from the Pentex Company (Kankakee, Illinois).

Brown fat cells were isolated by the procedure of Fain, Reed, and Saperstein (3) after digestion of minced brown adipose tissue from four or more rats which was divided between a bottle containing trypsin and one without. Two milliliters of phosphate buffer containing 4% albumin and 1 mg of collagenase per ml were present in each bottle. The albumin preparations referred to as A-27 and A-709 albumin were Lots D-27008 and E-30709, respectively, which were obtained from Armour. P-55 albumin refers to Lot P-55 obtained from the Pentex Company (Kankakee, Illinois).

Brown fat cells were isolated by the procedure of Fain, Reed, and Saperstein (3) after digestion of minced brown adipose tissue from four or more rats which was divided between a bottle containing trypsin and one without. Two milliliters of phosphate buffer containing 4% albumin and 1 mg of collagenase per ml were present in each bottle. The albumin preparations referred to as A-27 and A-709 albumin were Lots D-27008 and E-30709, respectively, which were obtained from Armour. P-55 albumin refers to Lot P-55 obtained from the Pentex Company (Kankakee, Illinois).

In all studies, except those in which oxygen consumption was
measured, the fat cells were incubated in plastic culture tubes (17 x 100 mm) at 37° in a shaking water bath with air as the gas phase. The values are based on the changes during the incubation period over that of initial controls which were incubated for 5 min. The values reported for each experiment are based on the average of duplicate tubes except for those in which oxygen consumption was measured. Every experiment was replicated at least three times on as many days.

Oxygen consumption was measured in a Gilson respirometer with the use of siliconized side arm Warburg flasks. The flasks were gassed with 100% oxygen and equilibrated for 30 min prior to the experimental period. Hormones and agents were added after basal respiration was monitored for 1 hour.

Glycerol (4) and lactate (5) were determined enzymatically on 50-μl aliquots of the medium, and DPNH formation was measured with a filter fluorometer (American Instrument). The wave length of the incident light was 340 ± 20 μu, and the secondary filter transmitted light above 410 μu.

Free fatty acids were determined on the remainder of the incubation medium and cells by a modification of the procedure of Dole and Meineitz (6) in which hexane was substituted for heptane, and the hexane was evaporated prior to titration of the free fatty acids. Triglyceride content per tube was determined by saponification of a 1-ml aliquot of the hexane upper phase and determination of the total fatty acid content (6). Glucose conversion to carbon dioxide, glyceride-glycerol, and fatty acid was determined as previously described (2, 3).

The amount of cells added to each tube was determined from the triglyceride content, and all parameters for both brown and white fat cells are standardized and expressed per millimole of triglyceride.

Bovine growth hormone (NIH-GH-B6) was a gift of the Endocrinology Study Section of the National Institutes of Health; desamethasone (9α-fluoro-11β,17α,21-trihydroxy-16α-methyl-1, 4-pregnadiene-3,20-dione) of Merck; crystalline insulin of Eli Lilly; prostaglandin El of Upjohn; and "bound insulin" of Dr. Harry Antoniades of the Blood Research Institute, Inc. The "bound insulin" was isolated from human serum and had a specific activity of 0.6 μunits per mg as based on intraperitoneal assay. L-Epinephrine bitartrate, L-norepinephrine bitartrate, and dl-isoproterenol hydrochloride were obtained from Calbiochem and theophylline from Mallinckrodt. Synthetic oxytocin, Lot 76B-1910, 10 units per mg, or Lot 118B-0670, 224 units per ml were obtained from Sigma. The trypsin inhibitor was chicken egg white ovomucoid, which inactivated approximately 0.7 to 0.9 mg of trypsin per mg of inhibitor (Worthington). The trypsin was either twice crystallized, dialyzed salt-free, lyophilized and dl-isoproterenol hydrochloride, and dl-isoproterenol, and growth hormone plus glucocorticoid (Table I).

Trypsin inhibitor (egg white ovomucoid) was added to flasks containing theophylline to inactivate any traces of trypsin which might have adhered to the cells during the washing procedure. The presence of trypsin inhibitor did not affect the antilipolytic action of prostaglandin El (Table I). Trypsin-treated fat cells responded just as well, if not slightly better, to lipolytic agents such as theophylline, isoproterenol, and growth hormone plus glucocorticoid (Table I).

Insulin failed to stimulate the metabolism of glucose by fat cells isolated by digestion with trypsin in addition to bacterial collagenase (Fig. 1). In cells isolated by digestion with collagenase alone insulin was able to increase the metabolism of glucose (Fig. 1). The basal rate of glucose metabolism by trypsin-treated cells was only slightly lower than that of control cells (Fig. 1). The antilipolytic action of insulin was also abolished by trypsin digestion of white adipose tissue (Table I). However, trypsin treatment did not affect the antilipolytic action of prostaglandin El (Table I). Insulin failed to stimulate the metabolism of glucose by fat cells isolated by digestion with trypsin in addition to bacterial collagenase (Fig. 1). In cells isolated by digestion with collagenase alone insulin was able to increase the metabolism of glucose (Fig. 1). The basal rate of glucose metabolism by trypsin-treated cells was only slightly lower than that of control cells (Fig. 1). The antilipolytic action of insulin was also abolished by trypsin.
Inhibition of antilipolytic action of insulin by digestion of white adipose tissue with collagenase plus trypsin

Free white fat cells were isolated by digestion of tissue with collagenase (1 mg per ml) or with collagenase plus trypsin (1 mg per ml). The free cells (20 μmoles of triglyceride per flask) from fresh rats were incubated for 4 hours in 1.5 ml of 4% albumin (A-27) buffer without added glucose. The concentrations of the various agents were: dexamethasone, 0.016 μg per ml; growth hormone, 0.75 μg per ml; theophylline, 0.2 mM; trypsin inhibitor, 0.16 mg per ml; isoproterenol, 0.005 μg per ml; insulin, 0.16 munit per ml; and prostaglandin E1, 0.1 μg per ml. All agents were added at the start of the incubation period except for theophylline and isoproterenol, which were added as 3 hours. The basal values are shown as the means of five paired experiments, and the increments due to insulin and prostaglandin E1 as the means ± standard errors of the paired differences.

Table I

<table>
<thead>
<tr>
<th>Additions</th>
<th>Glycerol release in cells isolated by digestion with collagenase</th>
<th>Glycerol release in cells isolated by digestion with collagenase plus trypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Increment due to insulin</td>
</tr>
<tr>
<td>None</td>
<td>0.0</td>
<td>-26 ± 8</td>
</tr>
<tr>
<td>Theophylline</td>
<td>38</td>
<td>-26 ± 8</td>
</tr>
<tr>
<td>Theophylline + trypsin inhibitor</td>
<td>41</td>
<td>-20 ± 10</td>
</tr>
<tr>
<td>Theophylline + dexamethasone and growth hormone</td>
<td>53</td>
<td>-40 ± 9</td>
</tr>
<tr>
<td>Isoproterenol + dexamethasone and growth hormone</td>
<td>57</td>
<td>-34 ± 8</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>43</td>
<td>-30 ± 10</td>
</tr>
</tbody>
</table>

Fig. 2. Ability of medium containing insulin which had previously been incubated with trypsin-treated white fat cells to stimulate the metabolism of glucose by a fresh batch of cells isolated by collagenase treatment. Free fat cells (20 μmoles of triglyceride per flask) were incubated for 4 hours in 1 ml of medium containing 4% albumin (A-27) and uniformly labeled glucose-\(^{14}C\) (2.8 μC). Half of the medium in each tube was fresh albumin buffer which contained the labeled glucose and half was from the experiments in Table I. At the end of the experiments shown in Table I, 0.5 ml of the medium which had been incubated with fat cells isolated by collagenase digestion (left) or by digestion with trypsin plus collagenase (right) was taken and frozen. The 0.5 ml of glucose-free albumin buffer from the previous experiments contained 0.1 μmole of theophylline (THEO) or 0.0025 μg of isoproterenol (ISO) both without and with insulin, 0.08 munit. The tubes containing 0.5 ml of buffer from the previous experiments were thawed and mixed with 0.5 ml of fresh buffer containing the labeled glucose. There was no lipolysis during the second incubation due to other theophylline or isoproterenol as based on net changes in medium glycerol. The values shown in the figure are the means of five paired experiments and are for glucose conversion in micromoles per minute of triglyceride to fatty acid, glyceride-glycerol, and carbon dioxide.

1 Unpublished observations.
Glucose conversion to lactate was almost completely abolished by trypsin digestion, but the increment due to oxytocin was unaffected (Fig. 3).

When white fat cells were digested with trypsin in P-55 albumin instead of A-27 or A-709 albumin preparations, the response to insulin was not abolished. The P-55 albumin preparations appeared to be contaminated with substances which protect white fat cells from trypsin inactivation of insulin action. In the studies with brown fat cells there appeared to be little difference between the various lots of albumin, and trypsin digestion abolished the effects of insulin on brown fat cells isolated and incubated in P-55 albumin (Table III).

Previously we have described a procedure for the isolation of fat cells from brown adipose tissue (3). These cells resemble white fat cells in that catecholamines stimulate lipolysis, and insulin both increases glucose metabolism and decreases lipolysis (3). Brown fat cells differ from white fat cells since they have a very small response to protein hormones such as adrenocorticotrophic hormone or growth hormone which activate white fat cell lipolysis (3). Another marked difference between brown and white fat cells is that the fatty acids released as a result of activation of lipolysis by catecholamines or dibutyryl-3',5'-AMP increase respiration by brown fat cells through a mechanism which is dependent on the presence of K+ (10, 11).

The stimulation by insulin of glucose conversion to carbon dioxide, total lipid, and lactate by brown fat cells was abolished by trypsin digestion (Fig. 4). The basal metabolism of glucose was slightly reduced by trypsin treatment, but isoproterenol was still able to stimulate glucose conversion to lactate. The lipolytic action of isoproterenol was not affected by trypsin treatment since in the experiments shown in Fig. 4 the increment in glycerol release in micromoles per millimole of triglyceride was 36 in control and 44 in trypsin-treated cells.

Trypsin digestion of brown adipose tissue also abolished the antilipolytic action of insulin (Table III). The lipolytic response to artemenol was slightly higher in trypsin-treated cells (Table III). These results indicate that trypsin-treated brown fat cells respond normally to lipolytic agents and it is only the antilipolytic action of insulin which is abolished by trypsin treatment.

In brown and in white fat cells prostaglandin E1 is a potent antilipolytic agent (13). In both brown (Table III) and white fat cells (Table I) trypsin digestion did not affect the antilipolytic action of prostaglandin E1.

Since oxytocin did not stimulate the metabolism of glucose by brown fat cells (Fig. 4), it was impossible to determine any effect of trypsin treatment on its mechanism of action. Studies with higher and lower concentrations also failed to detect any effect of this hormone on the metabolism of brown fat cells.

The stimulation of respiration due to addition of sodium octanoate to brown fat cells was significantly enhanced by insulin, and this action of insulin was also abolished by trypsin digestion. Trypsin digestion did not affect the basal rate of respiration by

### Table II

Failure of trypsin treatment to abolish stimulation of glucose metabolism by oxytocin.

<table>
<thead>
<tr>
<th>Glucose conversion to</th>
<th>Glucose converted in cells isolated with collagenase only</th>
<th>Glucose converted in cells isolated with collagenase plus trypsin, 1.5 mg per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Increment due to insulin</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>2.0</td>
<td>+2.0 ± 0.8</td>
</tr>
<tr>
<td>Glyceride-glycerol</td>
<td>2.2</td>
<td>+1.7 ± 0.7</td>
</tr>
<tr>
<td>Fatty acid</td>
<td>1.0</td>
<td>+3.4 ± 1.0</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.8</td>
<td>+6.1 ± 1.8</td>
</tr>
</tbody>
</table>

### Table III

<table>
<thead>
<tr>
<th>Glucose conversion to triglyceride</th>
<th>Glucose converted in cells isolated with collagenase only</th>
<th>Glucose converted in cells isolated with collagenase plus trypsin, 1.5 mg per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Increment due to insulin</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>2.0</td>
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<td>Glyceride-glycerol</td>
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<td>1.0</td>
<td>+3.4 ± 1.0</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.8</td>
<td>+6.1 ± 1.8</td>
</tr>
</tbody>
</table>
Cells were obtained by digestion of brown adipose tissue from fasted rats with 1 mg of collagenase per ml without and with 1 mg of trypsin per ml. Approximately 8 μmoles of triglyceride per flask in each group were incubated for 4 hours in 3 ml of medium containing 4% albumin (P-55), 2.8 mM glucose, and 0.1 mg of trypsin inhibitor per ml. The basal values are the means of seven paired experiments and the increments due to 0.17 munit of insulin per ml and 0.03 μg of prostaglandin E₁ per ml are the means ± standard errors of the paired differences. Arterenol (5 × 10⁻⁴ M) and sodium octanoate (2 mM) were added 1½ hours after the start of the incubation.

**Table III**

<table>
<thead>
<tr>
<th>Measurement and additions</th>
<th>Cells isolated with collagenase only</th>
<th>Cells isolated with collagenase and trypsin, 1 mg per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal increment due to insulin</td>
<td>Basal increment due to prostaglandin E₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increment due to insulin</td>
</tr>
<tr>
<td></td>
<td>μmole triglyceride</td>
<td>μmole triglyceride</td>
</tr>
<tr>
<td>Oxygen consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>12 ± 1</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Arterenol</td>
<td>70 ± 10</td>
<td>80 ± 16</td>
</tr>
<tr>
<td>Octanoate</td>
<td>80 ± 17</td>
<td>14.7 ± 6.9</td>
</tr>
<tr>
<td>Change in medium free fatty acid content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>12 ± 2</td>
<td>14 ± 7</td>
</tr>
<tr>
<td>Arterenol</td>
<td>100 ± 36</td>
<td>290 ± 88</td>
</tr>
<tr>
<td>Octanoate</td>
<td>5 ± 1.5</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>Glycerol release</td>
<td>37 ± 12</td>
<td>66 ± 12</td>
</tr>
<tr>
<td>None</td>
<td>10 ± 4</td>
<td>5 ± 2</td>
</tr>
</tbody>
</table>

* Over last 3 hours.

**Fig. 4.** Failure of oxytocin to stimulate glucose metabolism by brown fat cells. Brown adipose tissue from fed rats was digested with 1 mg of collagenase per ml in the absence and presence of 1.5 mg of trypsin per ml. The cells (10 μmoles of triglyceride per flask) were incubated for 4 hours in 1.5 ml of medium containing 4% albumin (A-709), 0.1 mg of trypsin inhibitor per ml, and 2.8 mM glucose-1°C. The values are shown as the means of three paired experiments and represent glucose conversion per μmole of triglyceride to carbon dioxide, total lipids, and lactate. Lactate formation was based on an enzymatic analysis of medium lactate. Insulin, 0.17 munit per ml; oxytocin, 0.5 unit per ml; and isoproterenol, 0.015 μg per ml, were present from the start of the experiment.

**DISCUSSION**

Northrop (13) reported in 1926 that living organism were resistant to digestion by trypsin, in contrast to dead organisms, which were readily digested by proteolytic enzymes. More recently De Luca (14) has shown that resistance to trypsin digestion can be used for the determination of viability in tissue culture cells. Cells which had been irreversibly damaged by toxic agents were readily digested by trypsin, and this was a more sensitive index of irreversible damage than uptake of vital dyes by damaged cells (14). Kono (1) found that pieces of white adipose tissue which had been incubated with trypsin appeared to be unaffected except that the response to insulin was abolished.

Digestion of tissues with trypsin or collagenase or both is a procedure used to obtain suspensions of cells for cell culture experiments (15). Rodbell (2) first reported that suspensions of fat cells which respond to insulin and other hormones could be obtained by digestion of white adipose tissue with bacterial collagenase. Fain, Reed, and Saperstein (3) found that suspensions of free brown fat cells can be obtained after digestion of minced brown adipose tissue with bacterial collagenase and these adipocytes respond to insulin. The present studies indicate that digestion of adipose tissue with trypsin in addition to collagenase specifically inactivates the response of fat cells to insulin and insulin-like substances such as "bound insulin."

The failure of trypsin-treated cells to respond to insulin was not due to inactivation of the insulin in the medium. In all our experiments a concentration of insulin was used (165 μunits per ml) which should have been considerably above that required to obtain maximal effects of insulin. The biological activity of this concentration of insulin was unaffected by incubation with trypsin-treated white fat cells for 4 hours (Fig. 2). In our experiments buffer containing insulin stimulated glucose metabolism by brown fat cells or the stimulation of respiration by arterenol or octanoate (Table III).
by a fresh batch of fat cells to the same extent after incubation with trypsin-treated cells as after incubation with fat cells isolated with collagenase alone. Kono has reported a similar finding in studies with intact adipose tissue (1). The present studies with suspension of free brown and white fat cells and those of Kono (1) with intact white adipose tissue clearly demonstrate that the effect of trypsin treatment is not on the inactivation of insulin but rather on the ability of cell receptors to react with it. Since “bound insulin” did not increase glucose metabolism by brown fat cells, it is clear that either brown fat cells are not able to release the insulin present in “bound insulin” or the insulin-like effects of this material are not due to insulin at all. With our present data we cannot disprove the hypothesis that white, but not brown fat cells, are able to release insulin from “bound insulin.” But if this process involves a proteolytic digestion of “bound insulin,” then the enzymes involved are insensitive to inhibitors of general proteolytic activity such as chicken egg white ovomucoid (Fig. 3) or preparations of pancreatic trypsin inhibitors of general proteolytic activity such as chicken egg white ovomucoid (Fig. 3) or preparations of pancreatic trypsin inhibitor prepared according to Kunitz and Northrop. We prefer the hypothesis that the activity of so-called “bound insulin” preparations is due to the presence of substance(s) derived from insulin which react with the insulin receptor of white but not brown fat cells, which is supported by the recent finding that “bound insulin” and insulin both disappear from the blood after “total” pancreatectomy of rats.

The hypothesis that the primary action of trypsin is to selectively inactivate the receptors for insulin and insulin-like substances rather than to inhibit the metabolic processes of fat cells is supported by the studies with prostaglandin E₁ and oxytocin. Trypsin-treated white or brown fat cells responded just as well to the antilipolytic action of prostaglandin E₁ as did cells isolated by digestion with collagenase alone. These data suggest that the antilipolytic actions of insulin and prostaglandin E₁ occur by quite distinct processes even though the final step in the action of both agents appears to be inhibition of adenyl cyclase activity (12, 16).

The failure of trypsin treatment to abolish the stimulation of glucose metabolism by oxytocin is of interest since both hormones have disulfide bridges and are polypeptides. However, the present results indicate that their mechanisms of action are quite different. Pilska et al. (17) have shown that the replacement of one or both of the sulfurs in oxytocin by methylene groups reduced but did not abolish the antidiuretic activity of oxytocin. Their data indicated that the antidiuretic effect of oxytocin does not involve some type of disulfide interchange between oxytocin and the hormone receptor. Whether the insulin-like effects of oxytocin on white fat cells also do not depend on disulfide groups remains to be demonstrated. The present results do indicate that the mechanism by which oxytocin stimulates glucose metabolism of white fat cells is quite distinct from that of insulin or insulin-like substances. The failure of oxytocin to stimulate glucose metabolism by brown fat cells indicates another difference between the hormonal responsiveness of brown and white fat cells. Since the brown cells appear to respond quite specifically to insulin, they should be more suitable for studies of the relationship between structure and activity of insulin derivatives and the mechanism of insulin action than white fat cells.

The effects of digestion of brown or white adipose tissue with relatively large amounts of trypsin (1 or 1.5 mg per ml) are quite different from what is seen with small amounts of trypsin. Kuo (18) found that incubation of white fat cells in the presence of 0.01 mg or less of trypsin or other proteolytic enzymes per ml resulted in a stimulation of glucose metabolism and inhibition of the lipolytic action of norepinephrine. Similar insulin-like effects can be obtained by addition of very small amounts of phospholipases to intact fat cells (19). Higher concentrations of the enzymes resulted in marked declines in glucose metabolism and rupture of the fat cells (18, 19). In our studies trypsin was never present during the incubation period, and to date we have failed to see any increase in basal glucose metabolism of cells isolated by digestion with 1 mg or more of trypsin per ml. The trypsin-treated fat cells were quite stable, and little lysis was seen over a 4-hour incubation period. Survey electron micrographs of brown fat cells did not reveal any apparent differences between the cells isolated by digestion with trypsin plus collagenase and those by digestion with collagenase alone.

The finding that the lipolytic action of growth hormone and glucocorticoid on white fat cells was unaffected by trypsin suggests that any postulated mechanism of growth hormone action involving an anti-insulin effect of the hormone is most unlikely. The lack of inhibition by trypsin of the lipolytic action of growth hormone and glucocorticoid is of interest since the mechanism of action of these hormones is different from that of other lipolytic agents. The onset of growth hormone and glucocorticoid action is much slower than that of the other known lipolytic agents and is blocked by inhibitors of RNA and protein synthesis such as actinomycin, puromycin, cycloheximide, X-ray, and ultraviolet light (20-22). The action of growth hormone and glucocorticoid is similar to that of catecholamines in that the protein or proteins made under the influence of growth hormone appear to affect the formation of cyclic nucleotide in white fat cells (23). Catecholamines activate lipolysis by increasing the formation of cyclic 3',5'-adenosine monophosphate from ATP, which is catalyzed by adenyl cyclase (24). The action of catecholamines differs from that of growth hormone in that it is rapid in onset and unaffected by inhibitors of RNA and protein synthesis (20-22).

The data presented here indicate that digestion of adipose tissue with trypsin specifically inactivates the response to insulin of both brown and white fat cells. The effects of trypsin do not appear to be due to interference with general cellular metabolism since the actions of other hormones are unaffected by trypsin digestion. Apparently a trypsin-sensitive component is involved in the mechanism of insulin action on both brown and white fat cells. Further studies will be required to elucidate the mechanism by which trypsin inactivates the insulin receptor.

Acknowledgment—We express our appreciation for the technical assistance of Doris Delekta.

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J. Biol. Chem. 1969, 244:3500-3506.

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