Adenyl Cyclase in Fat Cells

III. STIMULATION BY SECRETIN AND THE EFFECTS OF TRYPsin ON THE RECEPTORS FOR LIPOLYTIC HORMONES

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SUMMARY

The similarity in the chemical structures of secretin and glucagon prompted an investigation of the comparative effects of these hormones on adenyl cyclase activity in ghosts of rat fat cells and in a preparation of purified plasma membranes from rat liver.

Secretin did not activate adenyl cyclase or inhibit the stimulatory effects of glucagon on an adenyl cyclase system in plasma membranes of rat liver.

Secretin and glucagon stimulated adenyl cyclase activity in ghosts of fat cells. Secretin was a more potent stimulator of the enzyme; concentrations of secretin and glucagon giving half-maximal activities were, respectively, 0.1 and 0.3 µg per ml, and maximal activity given by secretin was about twice that given by glucagon. Secretin was also more effective than glucagon in stimulating lipolysis in isolated fat cells.

Combination of secretin and glucagon at maximal concentrations (20 µg per ml) did not give additive adenyl cyclase activities in ghosts, indicating that the hormones activate the same enzyme system in fat cells.

Ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid, a chelator of calcium, abolished the effects of adrenocorticotropin (ACTH) on adenyl cyclase in ghosts but did not inhibit the effects of secretin, glucagon, thyroid-stimulating hormone, and luteinizing hormones. 1-(2,4-Dichlorophenyl)-1-hydroxy-2-(t-butylamino)ethane hydrochloride, a β-adrenergic agent, stimulated adenyl cyclase and inhibited the effects of epinephrine. DCB did not inhibit the effects of secretin, glucagon, and ACTH.

Pretreatment of adipose tissue with trypsin decreased by 44% the lipolytic response of isolated fat cells to glucagon, but it had no effect on their response to secretin and ACTH. Glucagon did not stimulate adenyl cyclase activity in ghosts prepared from trypsin-treated fat cells; the effects of secretin and ACTH on the enzyme were reduced by 60 and 40%, respectively. Trypsin treatment had no effect on the activation of adenyl cyclase by fluoride ion and epinephrine.

It is concluded that secretin activates adenyl cyclase in fat cells via a receptor that is distinct from the receptors for glucagon and the other lipolytic hormones. It is suggested that the receptors for the peptide hormones are protein in character and are localized on the outer surface of the plasma membrane of fat cells.

EXPERIMENTAL PROCEDURES

Female Charles River rats (130 to 150 g, fed ad libitum) were used throughout these studies. Liver plasma membranes were

1 The abbreviations used are: ACTH, adrenocorticotropin; TSH, thyroid-stimulating hormone; LH, luteinizing hormone; NCH, 1-(2,4-Dichlorophenyl)-1-hydroxy-2-(t-butylamino)ethane hydrochloride; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid; cyclic AMP, cyclic adenosine 3',5'-monophosphate.
prepared according to the procedure of Neville (14). Preparation of trypsin-treated fat cells was based on the procedure described by Fain and Loken (15), in which trypsin was added during the digestion of adipose tissue with bacterial collagenase.

**Preparation of Free Fat Cells**—Cut pieces of parametral adipose tissue from four rats were distributed among four 1-oz plastic bottles containing 3.0 ml of 1% albumin-buffer and 1.0 mg per ml of collagenase. The composition of the buffer was: 124 mM NaCl, 4.0 mM KCl, 2.0 mM KH2PO4, 1.0 mM CaCl2, 1.5 mM MgCl2, and 25 mM Tris-HCl, pH 7.4. The pH of this buffer was readjusted with 2 M Tris after the addition of albumin. Incubation was carried out for 1 hour at 37°C in a shaking water bath. At the end of this period, the cells were filtered through a silk screen (13) and washed four times with 1% albumin-buffer.

For the preparation of trypsin-treated fat cells, the concentration of albumin during the collagenase treatment was increased to 3%, and trypsin was added to give a final concentration of 1 mg per ml. Soybean “trypsin inhibitor” (0.1 mg per ml) was added to the 1% albumin-buffer used for washing the cells. Free fat cells obtained from untreated adipose tissue were washed with the same medium.

**Preparation of Fat Cell “Ghosts”**—Fat cells isolated as described above were prepared from adipose tissue of 8 to 12 animals. The cells were lysed, and the resultant ghosts were isolated and washed as described previously (11). Ghosts were suspended in 1 mM KHCO3 to give between 5.0 and 8.0 mg of protein per ml and were assayed for their content of adenyl cyclase activity within 1 hour of preparation.

**Lipolytic Studies**—Free fat cells (containing 7 to 10 mg of triglyceride) were incubated at 37°C in a shaking water bath in a final volume of 2.0 ml of 1% albumin-buffer. Glycerol produced by the cells was determined enzymatically (16) on 0.2-ml aliquots of the incubation medium and is expressed per gram of cell triglyceride per hour. In those experiments in which the actions of trypsin were studied, 0.1 mg of soybean trypsin inhibitor was added per ml of incubation medium.

**Adenyl Cyclase Activity**—The activities of adenyl cyclase in ghosts of fat cells and in purified plasma membranes of liver cells were determined under incubation conditions (30°C, ATP-regenerating system present) and according to an assay procedure described previously (9, 17). In those experiments in which the actions of trypsin were studied, 0.1 mg of trypsin inhibitor was added per ml of incubation medium. The trypsin inhibitor had no effect on adenyl cyclase activity or its response to hormones. Adenyl cyclase activity is expressed as nanomoles of cyclic AMP formed in 10 min per mg of protein. Protein was determined by the Lowry procedure (18) with crystalline bovine albumin as standard.

**Materials**—Collagenase (type CLS, 150 units per mg), soybean trypsin inhibitor, and trypsin (twice crystallized) were purchased from Worthington; glucagon (crystalline) and DCB were supplied by Eli Lilly; highly purified secretin (porcine) was a generous gift from Dr. Victor Mutt (Karolinska Institute, Stockholm). Other hormones, reagents, and radiochemicals were the same as described in our recent publications (9, 11, 17).

**RESULTS**

It has been shown by others (6, 7) that secretin stimulates lipolysis (glycerol production) in adipose tissue of the rat. As shown in Table I, secretin also stimulated lipolysis in isolated fat cells obtained by collagenase treatment of rat adipose tissue. At concentrations of 0.01 and 0.5 μg per ml, secretin was more effective than glucagon as a lipolytic agent. Secretin stimulated adenyl cyclase activity in ghosts of fat cells and, as in the studies on lipolysis, was more potent than glucagon as a stimulator of the enzyme (Fig. 1). In three experiments, half-maximal adenyl cyclase activity was attained with 0.1 μg of secretin per ml and with 0.3 μg of glucagon per ml. At maximal concentrations of 20 μg per ml, secretin was about twice as effective as glucagon (Fig. 1) and was slightly less effective than ACTH (Table VII) in stimulating the enzyme.

It was previously shown (11, 12) that combinations of maximal concentrations of the lipolytic hormones (glucagon, ACTH, epinephrine, LH, and TSH) did not give additive adenyl cyclase activities, indicating that these hormones activate a common adenyl cyclase in fat cells. Similarly, combination of maximal concentrations (20 μg per ml) of secretin and glucagon did not give additive activities of adenyl cyclase (Table II), indicating

**Table I**

<table>
<thead>
<tr>
<th>Hormone added</th>
<th>Concentration (μg/ml)</th>
<th>Change in glycerol production (lipolysis) due to hormones (μmol/g cells/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon</td>
<td>0.01</td>
<td>12 ± 2</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>Secretin</td>
<td>0.01</td>
<td>19 ± 3</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>31 ± 3</td>
</tr>
</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>Hormone added</th>
<th>Concentration (μg/ml)</th>
<th>Change in adenyl cyclase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretin</td>
<td>0.01</td>
<td>0.1 nmole/g cells/hour</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.3 nmole/g cells/hour</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of various concentrations of secretin and glucagon on adenyl cyclase activity of fat cell ghosts. Fat cell ghosts (70 μg of protein) were incubated at 30°C for 10 min in a medium (final volume, 0.05 ml) containing 3.2 mM ATP-α-32P (Tris salt, pH 7.4, 35 cpm per pmole); 5 mM MgCl2; 10 mM theophylline; 20 mM creatine phosphate (Tris salt, pH 7.4); 25 mM Tris-HCl, pH 7.4; 1 mg per ml of creatine kinase (30 units per mg); 0.1% albumin; and the indicated additions of secretin and glucagon. The amount of cyclic AMP produced during incubation was determined according to Krishna, Weiss, and Brodie (19) as described by Rodbell (13). Adenyl cyclase activity is expressed as nanomoles of cyclic AMP formed in 10 min per mg of protein.
Adenyl Cyclase in Fat Cells. III

that secretin activates the same adenyl cyclase as does glucagon. These results also suggest that secretin and glucagon did not interact competitively with the enzyme. It was calculated, by the equations of Reiner (20), that competitive interaction of the two hormones should give an activity of 2.17; the activities found for the combined hormones were significantly higher than 2.17.

**Table II**

Effect of combination of maximally stimulating concentrations of glucagon and secretin on adenyl cyclase activity in ghosts of fat cells

Ghosts of fat cells were incubated as described in the legend to Fig. 1. Each hormone was added to give a final concentration of 10 μg per ml. Values are the means ± S.E. of duplicate demonstrations.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Change in adenyl cyclase activity due to hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon</td>
<td>1.10 ± 0.02</td>
</tr>
<tr>
<td>Secretin</td>
<td>2.53 ± 0.05</td>
</tr>
<tr>
<td>Glucagon + secretin</td>
<td>2.41 ± 0.06</td>
</tr>
</tbody>
</table>

**Table III**

Effect of glucagon and secretin on adenyl cyclase activity in liver plasma membranes

Liver plasma membranes (20 μg of protein) were incubated under the same conditions described in the legend to Fig. 1 with the exception that albumin and theophylline were omitted from the incubation medium. Hormones were added to give a final concentration of 10 μg per ml. Values are the means of two experiments ± S.E.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Change in adenyl cyclase activity due to hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon</td>
<td>2.12 ± 0.06</td>
</tr>
<tr>
<td>Secretin</td>
<td>0.63 ± 0.04</td>
</tr>
<tr>
<td>Glucagon + secretin</td>
<td>2.23 ± 0.10</td>
</tr>
</tbody>
</table>

**Table IV**

Effects of EGTA on stimulatory effects of ACTH, glucagon, secretin, TSH, and LH on adenyl cyclase activity in fat cell ghosts

Adenyl cyclase activity was measured at 30° in the incubation medium described in the legend to Fig. 1. EGTA was added as the magnesium salt to give a final concentration of 1 mM. Hormones were added to give a final concentration of 20 μg per ml. Values are the means of duplicate determinations ± S.E. Basal activity was not significantly changed by the addition of EGTA.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Change in adenyl cyclase activity due to hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-EGTA</td>
</tr>
<tr>
<td></td>
<td>+EGTA</td>
</tr>
<tr>
<td>ACTH</td>
<td>1.82 ± 0.40</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0.80 ± 0.10</td>
</tr>
<tr>
<td>Secretin</td>
<td>1.43 ± 0.20</td>
</tr>
<tr>
<td>TSH</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>LH</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>ACTH</td>
<td>1.26 ± 0.60</td>
</tr>
<tr>
<td>TSH</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>ACTH</td>
<td>1.90 ± 0.20</td>
</tr>
<tr>
<td>LH</td>
<td>0.20 ± 0.02</td>
</tr>
</tbody>
</table>

**Table V**

Effects of DCB on adenyl cyclase activity and response to epinephrine and secretin in fat cell ghosts

Adenyl cyclase activity in ghosts was measured according to the conditions described in the legend to Fig. 1. Values are the means ± S.E. of the two experiments.

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Concentration of DCB</th>
<th>Adenyl cyclase activity</th>
<th>Change due to hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0.23 ± 0.10</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>2.29 ± 0.06</td>
<td>0.45 ± 0.10</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>200</td>
<td>0.62 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0</td>
<td>0.29 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>0.2 μg/ml</td>
<td>2</td>
<td>0.16 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.08 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretin</td>
<td>0</td>
<td>0.86 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.84 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.80 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>0.20 ± 0.02</td>
<td>0.74 ± 0.08</td>
<td></td>
</tr>
</tbody>
</table>
carried out on the effects of pretreatment of adipose tissue with trypsin (1 mg per ml during collagenase treatment) on the actions of the hormones on lipolysis in fat cells and on adenylyl cyclase in ghosts. In order to minimize the possibility that trypsin was present in an active form during incubation of fat cells or ghosts, isolated fat cells were washed thoroughly with 1.0% albumin-buffer in the presence of trypsin inhibitor; the inhibitor was also incorporated in the incubation media used for studies of lipolysis and adenylyl cyclase activity. All studies were carried out in the presence of maximal concentrations of the hormones so that changes in hormone response would reflect alterations in the maximal activity of the receptor-adenyl cyclase system.

**Table VI**

**Effects of trypsin treatment of adipose tissue on effects of glucagon, secretin, ACTH, and epinephrine on lipolysis (glycerol production) in isolated fat cells**

Fat cells were isolated from adipose tissue treated or not with trypsin (1 mg per ml) during digestion of tissue with collagenase, as described under “Experimental Procedures.” The cells (7 to 12 mg per flask) were incubated for 2 hours in 1% albumin buffer containing 0.1 mg of soybean trypsin inhibitor per ml and the hormones at a concentration of 0.5 μg per ml. Values are the means ± S.E. of two experiments.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Change in lipolysis due to hormone</th>
<th>Effect of trypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmoles glycerol/g fat cell/hr</td>
<td>%</td>
</tr>
<tr>
<td>Glucagon</td>
<td>22 ± 2</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Secretin</td>
<td>30 ± 2</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>ACTH</td>
<td>40 ± 2</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>38 ± 2</td>
<td>39 ± 4</td>
</tr>
</tbody>
</table>

a Trypsin had no significant effect on basal lipolysis, which was 2.4 ± 0.5 μmoles of glycerol per g of fat cell per hour.

b Not significant.

**Table VII**

**Effects of treatment of fat cells with trypsin on actions of glucagon, secretin, ACTH, epinephrine, and fluoride ion on adenylyl cyclase activity in ghosts of fat cells**

Procedures for treatment of adipose tissue with trypsin and isolation of fat cells and ghosts are described in “Experimental Procedures.” Adenylyl cyclase activity in ghosts was measured under the same procedures described in the legend of Fig. 1. Hormones were added to give a concentration of 20 μg per ml; NaF was present at a concentration of 10 mM. Values are the means of two experiments ± S.E.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Change in adenylyl cyclase activity due to additions</th>
<th>Effect of trypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmoles cAMP/g fat cell/hr</td>
<td>%</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0.84 ± 0.02</td>
<td>0.06 ± 0.03b</td>
</tr>
<tr>
<td>Secretin</td>
<td>1.46 ± 0.02</td>
<td>0.05 ± 0.04</td>
</tr>
<tr>
<td>ACTII</td>
<td>1.56 ± 0.01</td>
<td>0.98 ± 0.05</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>1.55 ± 0.03</td>
<td>1.38 ± 0.11</td>
</tr>
<tr>
<td>NaF</td>
<td>2.28 ± 0.07</td>
<td>2.02 ± 0.05</td>
</tr>
</tbody>
</table>

a Basal activity, 0.30 ± 0.01, was unchanged by trypsin treatment.

b Not significant from basal activity.

The lipolytic effect of glucagon was reduced by 44% in fat cells pretreated with trypsin relative to untreated fat cells; the lipolytic effects of secretin and ACTH were not affected significantly in trypsin-treated fat cells (Table VI).

Ghosts were isolated from fat cells prepared from adipose tissue treated or not with trypsin in the same manner as described above. A comparison was made of the responsiveness of their adenylyl cyclase to hormones and to NaF, which also stimulates adenylyl cyclase in ghosts (18). As shown in Table VII, trypsin treatment inhibited completely the effect of glucagon on adenylyl cyclase activity and reduced the effects of secretin and ACTH on adenylyl cyclase activity in ghosts by approximately 60 and 40%, respectively. The response of the enzyme to epinephrine was not changed significantly in ghosts from trypsinized cells. The stimulatory effect of fluoride ion, which activates the same enzyme as do the hormones but by a different process (17), was also diminished significantly in ghosts from trypsin-treated fat cells. In contrast, trypsin (20 to 100 μg per ml) destroys adenylyl cyclase and its response to hormones and fluoride ion when added during incubation of ghosts at 30°C in 1 mM KHCO₃, pH 7.4 (17). The lipolytic effects of the peptide hormones were not altered to the same extent by trypsin treatment as were their effects on adenylyl cyclase activity (compare Tables VI and VII). The basis of this apparent discrepancy may be that, though the levels of cyclic AMP may be reduced in trypsin-treated cells in response to ACTH and secretin, they are sufficient to stimulate the lipolytic process. It has been shown that a 50% fall due to insulin in the level of cyclic AMP produced by fat cells in response to epinephrine (plus caffeine) does not result in a decrease in the lipolytic response (23). It would appear, therefore, that the lipolytic response of fat cells to hormones does not accurately reflect changes in the receptor-adenyl cyclase system.
was abolished in ghosts of trypsinized fat cells. Defining the early step affected by trypsin as receptor sites for secretin and glucagon, the differential effects of trypsin show that the receptors for these hormones are different. This is also supported by the finding that the hormones did not act on the enzyme in a competitive fashion. The failure of secretin to activate adenyl cyclase in a preparation of liver membranes or to inhibit the effects of glucagon on this enzyme is consistent with the finding that the receptors for glucagon and secretin are different. Secretin also did not react with either the receptor for ACTH or epinephrine. Thus secretin can be added to the list of hormones (glucagon, ACTH, epinephrine, TSH, and LH) that activate a common adenyl cyclase in fat cells via receptors that have distinct characteristics for each of the hormones. It should be emphasized that the term “receptor” is an operational one and refers to sites that selectively react with the hormones and which account, therefore, for the specificity of hormone action on adenyl cyclase. Chemical identification of a receptor for the lipolytic hormones has not been described. With the knowledge that the receptors for the lipolytic hormones are distinct from one another, a major question is whether the receptors are part of the adenyl cyclase molecule or whether they are present in separate molecules that are capable, through a coupling mechanism, of activating adenyl cyclase. The available evidence does not permit us to differentiate between these possibilities.

The differential effects of trypsin on the receptors for the peptide hormones deserve further discussion since these effects appear to have implications for the cellular location of the adenyl cyclase system in fat cells and the positional relationship of the receptors to the catalytic process in this system. Other studies have shown that adenyl cyclase activity is enriched in ghosts (13) or in more purified preparations of the plasma membrane of fat cells (26). Trypsin, under the conditions used in this study, does not cause a loss of cellular integrity or in the response of fat cells to the metabolic effects of prostaglandin, oxtocin, epinephrine, theophylline, and growth hormone plus glucocorticoids (15). However, the metabolic effects of insulin are diminished (15, 26), which suggests that trypsin only affects processes in the plasma membrane to which it is exposed or which are susceptible to attack by this enzyme, such as the receptor-effector system for insulin (26). Accordingly, the de-structive effects of trypsin on the receptors for glucagon, secretin, and ACTH but not of the catalytic (fluoride-activated) or epinephrine-sensitive components of the adenyl cyclase system provide suggestive evidence that the receptor sites for the peptide hormones are protein in character and are localized on the external surface of the plasma membrane (and thus exposed to hormones and trypsin). It can be imagined that the catalytic component of this system, presumably linked in some manner to the receptors, is situated on the inner cytoplasmic side of the membrane where it is exposed to the substrate, ATP.

REFERENCES

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