An Effect of Insulin on Cyclic Adenosine 3':5'‐Monophosphate Phosphodiesterase Activity in Fat Cells*

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SUMMARY

Homogenates of rat fat cells were separated into three fractions by centrifugation: P₁ (sedimented at 10,000 × g for 7 min), P₂ (sedimented from the 10,000 × g supernatant after 20 min at 100,000 × g), and S (the 100,000 × g supernatant). All fractions contained adenosine 3':5'-monophosphate (cAMP) phosphodiesterase activity. The S fraction was enriched in higher $K_m$ (≈ 15 μM) phosphodiesterase and the P₁ fraction in low $K_m$ (≈ 0.2 μM) activity, although both exhibited two apparent Michaelis constants for cAMP. When assayed with 62 nM cAMP, the specific activity of P₁ was 2 to 3 times that of the unfractinated homogenate, and this fraction contained more than one-half of the total phosphodiesterase activity.

Incubation of fat cells with 1.0 milliunit per ml of insulin invariably increased the phosphodiesterase activity assayed in homogenates with <10 μM cAMP. The increment in activity was for the most part confined to the P₁ fraction, the specific activity of which was increased 71 ± 5.5% (mean ± S.E.) in 24 experiments. The specific activity of 5'-AMP nucleotidase in P₁ (which was 3 to 4 times that in the whole homogenate) was not altered by insulin. The effect on phosphodiesterase activity was essentially maximal after exposure of cells to insulin. 1 milliunit per ml, for 6 to 8 min.

It was not diminished by washing the cells four times without insulin but was completely reversed by incubation for 30 min without insulin after washing. Neither prostaglandin E₁, 2.8 μM, nor nicotinic acid, 10 μM, mimicked the effect of insulin on fat cell phosphodiesterase activity. The effect of insulin was prevented by anti-insulin serum and insulin treated with dithiothreitol was inactive.

When present in the phosphodiesterase assay, dithiothreitol, 0.0002% to 4.2 mM, increased the activity of both P₁ and S fractions isolated from control cells, whereas heparin, 8.4 to 420 ng per ml, and ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA), 0.05 to 2.0 mM, increased P₁ activity with little or no effect on the activity of the supernatant fraction. Dithiothreitol, heparin, and EGTA increased, and sodium deoxycholate, 25 to 850 μg per ml, inhibited the activity of P₁ fractions isolated from control and insulin-treated cells. Triton X-100, 0.0025 to 0.25%, was without effect. Incubation at 45° for 5 min reduced the phosphodiesterase activity of P₁ fractions from control and insulin-treated cells to the same level which was about 50% of the initial control value. The activities then were stable for at least 25 min at 45°.

Treatment of rats with dexamethasone for 20 hours decreased the phosphodiesterase activity of the isolated fat cells. The decrement in activity of whole homogenates was apparently accounted for by a loss of activity in the P₁ fraction. These observations are consistent with the view that the effects of insulin and corticosteroids on cAMP-mediated processes in fat cells may be the result of alterations in the activity of a membrane-associated phosphodiesterase which has a relatively high affinity for cAMP.

The so-called lipolytic hormones exert their effects by enhancing the activity of fat cell adenylate cyclase, thus increasing the intracellular concentration of cAMP. Insulin inhibits the effects of these hormones on lipolysis, probably by preventing or diminishing the accumulation of cAMP (2). The latter could be brought about either by decreasing the rate of cAMP formation or increasing its destruction. Loten and Sneyd (3) first reported enhanced activity of a phosphodiesterase with a relatively high affinity for cAMP in homogenates of fat cells incubated with insulin. We have found that a large portion of the low $K_m$ phosphodiesterase of fat cell homogenates is associated with a particulate fraction (1) and the increment in phosphodiesterase activity produced by insulin appears to be confined to this fraction. Some characteristics of the phosphodiesterase activity in fat cell homogenates are described below with emphasis on the low $K_m$ particulate enzyme and the nature of the effects of insulin on its activity. In addition, it is shown that the activity of what may be the same phosphodiesterase is decreased by treatment of rats with dexamethasone.

MATERIALS AND METHODS

Fat cells were prepared as previously described from epididymal fat pads of Osborne-Mendel rats weighing 125 to 200

The abbreviations used are: cAMP, adenosine 3':5'-monophosphate; PGE₁, prostaglandin E₁; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid; P₁, P₂, and S fractions of fat cell homogenates separated by centrifugation as described under "Materials and Methods."
g (4). Rats were allowed free access to food and water until food was removed about 18 hours before decapitation. Samples of cells were incubated (37°C in 4.0 ml of Krebs-Ringer phosphate medium containing bovine serum albumin, 30 mg per ml. After 10 min, insulin was added to some cells and the incubation continued, usually for 15 min. All cells were then washed twice with approximately 8 ml of medium without albumin and twice with similar volumes of 0.25 M sucrose containing 10 mM Tris, pH 7.4. Solutions for washing cells were kept at 37°C. In early experiments, insulin, 1 milliunit per ml, was included in the media used for washing the insulin-treated cells but in later experiments all cells were washed with the media described without other additions.

After washing, the cells, suspended in 5 ml of 0.25 M sucrose, 10 mM Tris, pH 7.4, were homogenized in a 7-ml Dounce homogenizer (eight strokes with a B pestle). A sample of each homogenate was stored in ice and the remainder was centrifuged at 10,000 × g for 7 min at 0–5°C. The fat cake was removed with a spatula, the supernatant decanted, and the sides of the tubes wiped dry, taking care not to disturb the pellet (P1). The supernatant was then centrifuged at 100,000 × g for 20 min, the supernatant (S) decanted, and the sides of the tube wiped dry, again taking care not to disturb the pellet (P2). Both the 10,000 × g pellet (P1) and the 100,000 × g pellet (P2) were suspended in 0.25 M sucrose, 10 mM Tris, pH 7.4, and dispersed using a Dounce homogenizer.

For assay of phosphodiesterase activity, samples were incubated at 30°C in a total volume of 0.3 ml containing 25 μmoles of Tris-HCl, pH 7.4, 8.3 μmoles of MgCl₂, 2 μmoles of [3H]cAMP (16.4 or 25.5 Ci per mmole), and unlabeled cAMP. The reaction was terminated by addition of 25 μmoles of HCl, 400 μmoles of cAMP, and 500 μmoles of 5'-AMP in 75 μl. After heating for 4 min at 70°C, 25 μmoles of NaOH and 25 μmoles of Tris, pH 8.0, in 50 μl were added, followed by 75 μl of 100 mM Tris buffer, pH 8.0, containing 0.18 mg of Crotilus adamanteus venom (Sigma). The incubation with 5'-nucleotidase proceeded for 30 min at 37°C after which the samples were diluted, centrifuged, and [3H]adenosine isolated for radioassay, as previously described (5). Under the conditions employed, less than 15% of substrate was hydrolyzed in 10 min and for many experiments the activities reported represent verified initial rates of hydrolysis. One microunit of phosphodiesterase activity equals 1 pmole of cAMP hydrolyzed per min, at 30°C. All samples were assayed in duplicate with at least two different concentrations of substrate, although in many instances only the activities with one concentration (62 nM cAMP unless otherwise stated) are reported. All experiments have been replicated at least twice with different preparations of cells.

Assay of 5'-AMP nucleotidase activity was carried out in a total volume of 0.3 ml containing 25 μmoles of Tris-HCl buffer, pH 7.4, 8.3 μmoles of MgCl₂, about 2 μmoles of 5'-[3H]AMP (24.9 Ci per mmole), and unlabeled 5'-AMP with incubation at 30°C. The reaction was terminated by addition of 25 μmoles of HCl, 500 μmoles of 5'-AMP, and 500 μmoles of adenosine in 75 μl. After 4 min at 70°C, 25 μmoles of NaOH and 25 μmoles of Tris buffer, pH 8.0, in 175 μl were added, and [3H]adenosine isolated for radioassay. One microunit of activity equals 1 pmole of 5'-AMP hydrolyzed per min at 30°C. Protein was determined by the method of Lowry et al. (6), using bovine serum albumin as standard.

Fig. 1. Subcellular distribution of phosphodiesterase activity assayed at three concentrations of cAMP. Total activity (specific activity × total amount of protein) of each fraction is expressed as a percentage of that recovered in the three fractions (P1 plus P2 plus S) which was >80% of that of the whole homogenate (W).

Amorphous porcine insulin (24 units per mg) was kindly supplied by Dr. J. Bromer of Eli Lilly and Co., PGE₀, by Dr. John Pike of the Upjohn Co., and dexamethasone (sodium phosphate salt) by Dr. W. Goll of Merck and Co. Guinea pig anti-porcine insulin serum (Catalogue No. A-20-300) was purchased from Arnl Products Co., dexamethasone (Decadron) from Merck and Co., [3H]cAMP and 5'-[3H]AMP from New England Nuclear, nucleotides from Sigma, QAE-A25 Sephadex* from Pharmacia, nicotinic acid and cycloheximide from Nutritional Biochemicals, ouabain and dithiothreitol from Calbiochem, sodium heparin U.S.P. from Hyson, Wescott & Dunn, EGTA from Eastman Kodak Co., sodium deoxycholate from Mann Research Laboratories, and Triton X-100 from Packard Instrument Co.

RESULTS

The distribution of phosphodiesterase activity in the three homogenate fractions, P₁ (sedimented at 10,000 × g for 7 min), P₂ (sedimented from the 10,000 × g supernatant after 20 min at 100,000 × g), and S (the 100,000 × g supernatant) is shown in Fig. 1. When assays were performed with 62 nM cAMP,

* In the experiments reported here, QAE-A25 columns were used. We have recently found, however, that the QAE-A25 columns do not permit complete recovery of inosine, whereas inosine and adenosine are recovered together quantitatively from DEAE-A25 columns. Under conditions of the phosphodiesterase assay, the formation of [3H]inosine by fat cell homogenates or fractions is minimal, and phosphodiesterase activities in fractions from control and insulin-treated cells are essentially identical whether DEAE or QAE columns are used. We nevertheless now use DEAE-A25 (Pharmacia) columns rather than QAE-A25.
the specific activity of $P_2$ was 2 to 3 times that of the original homogenate or the other two subcellular fractions and $P_2$ contained more than one-half of the total phosphodiesterase activity recovered in the three fractions. Less than 50% of the activity assayed with 1.1 $\mu$M cAMP, however, was recovered in $P_2$. When phosphodiesterase was assayed with 110 $\mu$M cAMP, the supernatant fraction exhibited the highest specific activity, and accounted for 80% of the total activity recovered in the three fractions. Although evidently enriched, respectively, in high and low $K_m$ phosphodiesterase activity, the $S$ and $P_2$ fractions each exhibit two apparent Michaelis constants for cAMP as shown in Fig. 2.

When assays were performed with 62 nm cAMP, the phosphodiesterase activity of whole homogenates was consistently elevated by incubation of fat cells with insulin, 1 milliunit per ml, for 15 min. As shown in Fig. 3, the percentage increase in specific activity was considerably greater in $P_2$ than it was in the whole homogenate, whereas the activities of the $P_1$ and $S$ fractions were usually changed little if at all. The distribution of homogenate protein among the three fractions was not altered by incubation treatment and the recoveries of protein and phosphodiesterase activity were always greater than 80%. Thus, essentially all of the insulin-induced increment in activity of the whole homogenate was recovered in the $P_2$ fraction (Table I). When 1.1 $\mu$M cAMP was used in the phosphodiesterase assay, the activity of the homogenate of the insulin-treated cells was only slightly greater than that of the control cells and with 11 $\mu$M cAMP as substrate, there was no difference between the homogenates (Fig. 3). An effect of insulin on the activity in $P_2$ was detected with assays at all three substrate concentrations but the percentage effect was smaller with the higher concentrations of cAMP. In four experiments in which 1 milliunit per ml of insulin increased the phosphodiesterase activity by 57.8 ± 8% (mean ± S.E.), 0.1 milliunit per ml produced an increase of 26.5 ± 5.9%. In two experiments, 0.01 milliunit per ml produced little or no increase in phosphodiesterase activity. Incubation of fat cells with 1 milliunit per ml of insulin produced an increase in the velocity of the $P_2$ phosphodiesterase activity with little or no change in the apparent $K_m$ (approximately 0.2 $\mu$M) for cAMP.

As shown in Table II (Experiment 1), exposure of fat cells to insulin for 6 to 8 min during the washing period increased phosphodiesterase activity to essentially the same level found in the cells incubated for 15 min with insulin before washing in the same way. Prolonging the period of exposure to 30 min plus 6 to 8 min of washing caused little or no further increase (Fig. 4). After incubation of cells for 15 min with insulin, washing four times in medium without insulin over a period of 6 to 8 min did not reverse the effect of insulin. When, however, the cells were incubated further for 30 min without insulin, the phosphodiesterase activities were not different from those of cells never exposed to insulin (Table II, Experiments 2 and 3).

Essentially all of the 5'-AMP nucleotidase activity of the fat cells were used and incubation for 45 to 60 min with 4 mg per ml of a different lot of collagenase was required to obtain adequate dispersion of fat cells.
cell homogenates was recovered in the $P_1$ and $P_2$ fractions, with the highest specific activity in the latter. Nucleotidase activity was not altered by incubation of fat cells with insulin (Table III). The specific activity of adenylate cyclase was somewhat ($<100\%$) higher in the $P_1$ fraction than it was in the $P_2$ fraction but the percentage effects of isoproterenol and NaF were similar in both. Adenylate cyclase activity (basal or in the presence of isoproterenol or NaF) was not altered by incubation of cells with insulin (data not shown).

Neither PGE$_1$ nor nicotinic acid mimicked the effects of insulin on fat cell phosphodiesterase activity. Addition of cycloheximide or ouabain to the incubation medium did not prevent or modify the increase in phosphodiesterase activity produced by insulin (data not shown). Anti-insulin serum, which, in this experiment but not in others, appeared to have a small effect, completely abolished the effect of insulin and insulin incubated with dithiothreitol for 15 hours before addition to fat cells produced no increase in phosphodiesterase activity (Table IV). When added to the phosphodiesterase assay, on the other hand, 42 or 420 $\mu$m dithiothreitol markedly increased phosphodiesterase activity in $P_2$ fractions from control and from insulin-treated

### Table II

**Effect of insulin on phosphodiesterase activity in $P_2$ fraction**

Fat cells were incubated for 15 min, and washed four times with or without insulin, 1 milliunit per ml, as indicated. In Experiments 1 and 2, they were then homogenized in, and fractions suspended in, medium without insulin. In Experiment 3, after the first four washes, the cells were incubated for 15 min in medium of the same composition as the last wash, then insulin or diluent was added as indicated in parentheses. After incubation for 15 min more the cells were washed four times without insulin and homogenized. Phosphodiesterase activities of whole homogenates and $S$, $P_1$, and $P_2$ fractions were assayed. Since the effect of insulin was essentially confined to the homogenate and $P_1$ fractions, only the values for $P_2$ phosphodiesterase activity are presented.

| Insulin present in | Phosphodiesterase activity |
|-------------------|-----------------------------|
| Incubation | Wash | microunits/mg protein |
| 1 | 0 | 0 | 61 |
| | + | + | 103 |
| | 0 | + | 93 |
| + | 0 | + | 83 |
| + | + | + | 119 |
| + | + | (0) | 68 |
| | | | |
| 2 | 0 | 0 | 33 |
| | + | + | 83 |
| 3 | 0 | 0 (0) | 70 |
| | 0 | 0 (+) | 124 |
| | + | + (+) | 119 |
| | | | |
| | | 0 (0) | 68 |

### Table III

**Effect of insulin on phosphodiesterase and 5'-nucleotidase activity**

Fat cells were incubated with or without insulin, 1 milliunit per ml, for 15 min. Enzyme activities were assayed at the substrate concentrations indicated.

| Homogenate fraction | Phosphodiesterase activity, 62 nM CAMP | 5'-Nucleotidase activity, 90 nM 5'-AMP | 166 $\mu$m 5'-AMP |
|---------------------|----------------------------------------|--------------------------------------|--------------------|
| Control | Whole | 22 | 23 | 4,300 |
| | $P_1$ | 13 | 38 | 7,470 |
| | $P_2$ | 65 | 68 | 16,000 |
| Insulin | Whole | 33 | 26 | 4,010 |
| | $P_1$ | 17 | 36 | 7,500 |
| | $P_2$ | 105 | 77 | 16,400 |

### Table IV

**Effect of anti-insulin serum and dithiothreitol-treated insulin on phosphodiesterase activity in $P_2$**

In Experiment 1, 0.5 ml of guinea pig anti-insulin (porcine) serum or 0.5 ml of buffer was added to flasks containing 3.5 ml of fat cell suspension. After incubation for 15 min, insulin, 1 milliunit per ml, was added to some of the flasks and incubation continued for 15 min. In Experiment 2, insulin, 24 units per ml, was incubated with or without 100 mM dithiothreitol for 15 hours at room temperature. Cells were incubated for 15 min with 1 milliunit per ml of native insulin, an equivalent concentration of the dithiothreitol-treated insulin, or an equivalent concentration of dithiothreitol (about 4 $\mu$m). All cells were washed in the media described under "Materials and Methods" with no additions. Phosphodiesterase activity was assayed with 62 nM and 1.1 $\mu$m cAMP.

| Addition | Phosphodiesterase activity |
|----------|-----------------------------|
|          | 62 nM CAMP | 1.1 $\mu$m CAMP |
|          | microunits/mg protein |
| Experiment 1 | None | 86 | 330 |
| | Insulin | 146 | 580 |
| | Anti-insulin serum | 112 | 420 |
| | Insulin plus antiserum | 86 | 380 |
| Experiment 2 | None | 47 | 160 |
| | Insulin | 86 | 330 |
| | Dithiothreitol | 46 | 160 |
| | Dithiothreitol-treated insulin | 42 | 170 |
cells. With 4.2 mM dithiothreitol, the stimulation of activity in the control P2 fractions was less and in some instances the effect of insulin was apparently at least partially reversed (Fig. 5). Dithiothreitol, 42 to 420 µg per ml, also increased activity in the S fraction from control cells (Fig. 6).

Addition to the assays of EGTA, 0.05 to 2 mM, or heparin, 42 to 420 µg per ml, increased phosphodiesterase activity in P2 fractions and the increments were similar with fractions from control and insulin-treated cells (Fig. 5). As shown in Fig. 6, these agents produced little or no stimulation of phosphodiesterase in the S fraction. In fact 0.02 mM EGTA was inhibitory when the S fraction was assayed with 1.1 µM cAMP. Sodium deoxycholate, at concentrations between 250 and 2500 µg per ml, inhibited P2 fractions from control and insulin-treated cells to roughly the same extent (Fig. 5), whereas Triton X-100, 0.0025 to 0.25%, was without effect.

As shown in Fig. 7, phosphodiesterase (assayed with 62 nM cAMP) was rapidly inactivated by incubation of the P2 fractions at 45°C. Within about 5 min the fractions from control and insulin-treated cells had reached the same specific activity which was about 50% of the initial control level and there was little further inactivation during the next 25 min at 45°C. (The activity remaining after 20 min at 45°C still exhibited two K_m values for cAMP.)

Treatment of rats with dexamethasone decreased the phosphodiesterase activity of the isolated fat cells. The effect, which was demonstrable only when assays were carried out with <10 µM cAMP, was essentially maximal within about 20 hours after the first injection of the steroid hormone. It was most marked in the P2 fraction (Fig. 8) and, in fact, the entire decrement in activity of the whole homogenates was apparently accounted for by the loss of activity in the P2 fraction. The phosphodiesterase activity of fat cells from rats treated with dexamethasone for 20 or 40 hours could still be enhanced by incubation of the cells with insulin (data not shown).

**DISCUSSION**

We have found, as did Loten and Sneyd (3), that incubation of fat cells with insulin, 0.1 to 1.0 milliunit per ml, for 6 to 8 min or less, produces an increase in the phosphodiesterase activity measured in homogenates of these cells using cAMP concentrations of less than about 10 µM (1, 3). Fat cell homogenates contain phosphodiesterase activity with more than one apparent Michaelis constant for cAMP (1, 3, 5) and more than one-half of the low K_m activity, i.e. the activity assayed with submicromolar concentrations of cAMP, is associated with particulate elements of the homogenate. The fraction referred to above as P2 (sedimented in 20 min at 100,000 × g after re-

![Fig. 5. Effect of dithiothreitol, EGTA, heparin, and sodium deoxycholate on phosphodiesterase activity in P2 fractions. Fractions from control (●) and insulin-treated (○) cells were assayed with 62 nM cAMP and other additions as indicated. Under conditions of the assay, sodium deoxycholate did not inhibit the 5'-AMP nucleotidase activity of the snake venom used in the phosphodiesterase assay.](http://www.jbc.org/)

![Fig. 6. Effect of dithiothreitol, EGTA, and heparin on phosphodiesterase activity in P2 and S fractions. P2 fractions (●) and S fractions (○) from control cells were assayed with 62 nM (—) and 1.1 µM (···) CAMP. Sodium deoxycholate, at concentrations between 250 and 2500 µg per ml, did not stimulate P2 activity.](http://www.jbc.org/)

![Fig. 7. Effect of incubation at 45°C on phosphodiesterase activity. The S fraction (●) from control cells, and P2 fractions isolated from control (○) cells, and cells incubated with insulin, 1 milliunit per ml, for 15 min (▲) were incubated in 0.25 M sucrose, 0.05 M Tris HCl, pH 7.4, at 45°C before assay at 30°C with 62 nM cAMP. P2 fractions from control (□) and insulin-treated cells (■) were also assayed after 30 min at 0°C.](http://www.jbc.org/)

![Fig. 8. Effect of incubation at 45°C on phosphodiesterase activity. The S fraction (●) from control cells, and P2 fractions isolated from control (○) cells, and cells incubated with insulin, 1 milliunit per ml, for 15 min (▲) were incubated in 0.25 M sucrose, 0.05 M Tris HCl, pH 7.4, at 45°C before assay at 30°C with 62 nM cAMP. P2 fractions from control (□) and insulin-treated cells (■) were also assayed after 30 min at 0°C.](http://www.jbc.org/)
The effect of insulin on a membrane fraction from rat liver has been reported (11), but from the data presented it is not clear whether insulin increased phosphodiesterase activity or prevented inactivation during the assay period.

The observations reported here are consistent with our view that at least one of the consequences of the interaction of insulin with its specific binding sites on the fat cell surface is an increase in the activity of a phosphodiesterase in the plasma membrane, a phosphodiesterase with a relatively high affinity for cAMP, positioned in close proximity to the adenylate cyclase.

Thus, degradation of cAMP could be regulated at a site very near that at which its synthesis takes place and is regulated.

The rapid and reversible effect of insulin on membrane phosphodiesterase activity would appear sufficient to account for its effects on fat cell cAMP content (2) and on lipolysis (8, 9). These could, of course, also result from an insulin-induced depression of adenylate cyclase activity. Although we and others have consistently failed to observe such an effect (11, 12), it has been reported that insulin can prevent or diminish the stimulatory effects of glucagon on adenylate cyclase activity from liver (13) and fat cells (14). Since the cell fractions in which adenylate cyclase activity is measured consist of more or less purified plasma membranes, the phosphodiesterase that is stimulated by insulin is presumably also present. To determine whether insulin can in fact modulate the synthesis as well as the degradation of cAMP in fat cells, it will be necessary to investigate the effects of insulin on an adenylate cyclase preparation that is free of phosphodiesterase activity.

It appears that the membrane-bound low K_m diesterase may also play a role in the action of corticosteroid hormones on cAMP-mediated processes in fat cells. Senft et al. (15) originally reported that treatment of adrenalectomized rats with 6-methylprednisolone decreased the elevated phosphodiesterase activity of adipose and several other tissues. The effect on liver phosphodiesterase activity was noted 15 hours after a single subcutaneous injection. As reported above, the decrement in fat cell phosphodiesterase activity observed after 10 to 40 hours of dexamethasone treatment in intact rats was apparently confined to the same particulate component of low K_m phosphodiesterase in which the acute effect of insulin is localized.

Thus far, our attempts to increase phosphodiesterase activity by the addition of insulin to homogenates or P_2 fractions before or during assay have been unsuccessful. Similarly, Carter and Martin (10) were unable, by the direct addition of insulin, to stimulate glucose uptake in a "microsomal" fraction from fat cells prepared by a procedure very similar to that used for preparation of P_2 although the microsomal fractions prepared from cells incubated with insulin took up glucose at an enhanced rate compared to that of fractions from control cells. Loten and Eneyd (3) also found that the addition of insulin to fat cell homogenates was without effect on phosphodiesterase activity. An effect of insulin on a membrane fraction from rat liver has been reported (11), but from the data presented it is not clear whether insulin increased phosphodiesterase activity or prevented inactivation during the assay period.

Acknowledgments—We thank Dr. Blair Bowers for the electron microscopy of material sedimented in 7 min at 10,000 \( \times g \) containing 50 to 60% of the low K_m activity with a specific activity about 3 times that of the homogenate. By electron microscopy this fraction appears to consist largely of smooth membranous structures arranged in vesicles and sheets. It contains adenylate cyclase and 5'AMP nucleotidase (enzymes presumed to be localized to the plasma membrane), the latter with a specific activity about 4 times that of the whole homogenate. Essentially all of the increase in low K_m phosphodiesterase activity produced by incubation of cells with insulin occurs in this fraction. About 50% of the low K_m phosphodiesterase activity in P_2 fractions from control cells and all of the insulin-induced increment was destroyed after 5 min at 45\(^\circ\)C while the remaining activity was stable for at least 30 min at this temperature. If by this treatment we have distinguished a heat-labile enzyme whose activity is enhanced by insulin from a heat-stable one whose activity is unaffected by insulin, it is apparent that insulin treatment may produce a 200% increase in the activity of this enzyme.

Heparin and EGTA markedly enhanced phosphodiesterase activity in P_2 fractions but not that in the supernatant. The stimulatory effects of heparin, EGTA, and dithiothreitol on phosphodiesterase in P_2 were of roughly similar magnitude whether this fraction was derived from control or insulin-treated cells, and there was no obvious difference in the susceptibility of the two types of P_2 fractions to inhibition by deoxycholate.

The effect of insulin on phosphodiesterase is specific in that it was prevented by anti-insulin serum and was not produced by insulin previously incubated with dithiothreitol. In addition, neither PGE_1 nor nicotinic acid in concentrations which are anti-lipolytic and which diminish the magnitude of the increase in cAMP content of fat cells exposed to epinephrine (1, 7), altered phosphodiesterase activity (assayed with 62 nM cAMP). It may be noted that the concentrations of insulin, 0.1 to 1.0 milliunit per ml, that increase fat cell phosphodiesterase activity are similar to those employed for inhibition of the effects of epinephrine and other hormones on lipolysis (1, 8, 9) and on the cAMP content of fat cells (2).
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