Effects of Prostaglandins on Adenosine 3′,5′-Monophosphate Levels in Fat and Other Tissues*

(Received for publication, November 1, 1967)

R. W. Butcher† and Christine E. Baird

From the Department of Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee 37203

SUMMARY
The action of prostaglandins on cyclic AMP levels in rat epididymal fat pads, isolated fat cells, and other tissues was studied.

Fat pads incubated with prostaglandin E1 (PGE1) contained higher levels of cyclic AMP than controls but lipolysis was not stimulated. However, PGE1 did not cause increased cyclic AMP levels in isolated fat cells, suggesting that the stimulatory action occurred in another cell type. PGE1 at very low concentrations antagonized the action of epinephrine on cyclic AMP levels in both intact fat pads and isolated fat cells.

PGE1 was considerably more effective as an inhibitor of epinephrine than PGF1α and PGE1 was without effect. PGE1 lowered cyclic AMP levels in isolated fat cells very rapidly, and was effective against adrenocorticotropic hormone, glucagon, and thyroid-stimulating hormone as well as the catecholamines.

Incubation of intact cell preparations of lung, spleen, diaphragm, kidney, and other tissues with 2.8 μM PGE1 resulted in increased levels of cyclic AMP.

Studies by Bergström and others have established the prostaglandins as compounds of great biological importance. The actions, occurrence, metabolism, and biosynthesis of the prostaglandins have been the subject of a recent symposium (1) and two reviews (2, 3), to which the interested reader is referred.

Effects of prostaglandins on the rat epididymal fat and the toad bladder suggested that cyclic AMP might be involved at least in part in their actions. First, Steinberg and Vaughan, working in association with Bergström, found the prostaglandin E1 antagonized the lipolytic actions of the catecholamines, adrenocorticotropic hormone, glucagon, and thyroid-stimulating hormone, and that the activation of phosphorylase by these hormones was also inhibited by PGE1. Since cyclic AMP had been implicated in the hormonal activations of lipolysis (2) and phosphorylase (6), it was conceivable that the prostaglandins acted in adipose tissue by altering cyclic AMP levels. Another action of PGE1 which seemed to involve cyclic AMP was reported by Orloff, Handler, and Bergström (7), who showed that the effects of vasopressin on water and ion movement in the toad bladder were antagonized by PGE1. Cyclic AMP, which mimicks the action of vasopressin on the toad bladder and which mediates the effect of the hormone (8), was not antagonized by PGE1.

In the experiments being reported, low concentrations of prostaglandins decreased intracellular cyclic AMP levels in isolated fat cells very rapidly and with considerable specificity. By contrast, incubation with PGE1 increased cyclic AMP levels in other tissues, including a component of whole fat pads, lung, spleen, diaphragm, and other tissues. A preliminary report of some of this work has been published (9).

EXPERIMENTAL PROCEDURE
In the experiments involving the incubation of intact fat pads, the pads were removed from young male rats under Nembutal anesthesia. They were divided into proximal, medial, and distal segments, and then distributed on this basis into 50-ml flasks containing 20 ml of Krebs-Ringer phosphate buffer with 200 μg per ml of albumin. The flasks were incubated for 24 min at 37°C, and then the tissues were transferred to fresh Krebs-Ringer phosphate buffer containing the desired additions. These flasks were incubated for an additional 24 min with shaking, and the experiment was terminated by removing the pieces of fat from the flasks and dropping them into an operating Waring Blendor containing 17 ml of 0.1 N HCl containing purified tritiated cyclic AMP (about 10,000 dpm). The homogenates were placed in a boiling water bath for 15 min and centrifuged at 8000 × g for 15 min, and the supernatant fractions were fractionated and cyclic AMP was analyzed as described in the accompanying report (10).

Isolated fat cells were prepared as described in the accompanying publication (10). Crystalline prostaglandins were obtained

* The research reported here has been supported in part by Grants AM-07462 and HE-08332 from the United States Public Health Service.
† Investigator of the Howard Hughes Medical Institute.

1 The abbreviations used are: PGE1 (prostaglandin E1), 11α,15-(S)-dihydroxy-9-oxo-13-trans-prostenoic acid; PGE1α, (prostaglandin F1α), 9α,11α,15-(S)-trihydroxy-13-trans-prostenoic acid; PGE1β (prostaglandin F1β), 9β,11α,15-(S)-trihydroxy-13-trans-prostenoic acid.
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FIG. 1. The effect of PGE1 on cyclic AMP levels in the rat epididymal fat pad in vitro. Fat pads were removed from young male rats under pentobarbital anesthesia. The pads were divided into proximal, medial, and distal segments, and then distributed on this basis into 50-ml flasks containing 20 ml of Krebs-Ringer phosphate buffer. The flasks were incubated for 24 min at 37°, and then the pads were transferred to fresh Krebs-Ringer phosphate buffer containing the additions as shown. PGE1 was prepared for use as described under "Experimental Procedure," and the dilution of the PGE1 was made in the control solution. These flasks were incubated for an additional 24 min with shaking, and the experiment was terminated by removing the pieces of fat pad from the flasks and dropping them into an operating Waring Blendor containing 17 ml of 0.1 M HCl containing tritiated cyclic AMP and boiled for 12 min. After centrifugation at 8000 × g for 15 min at 4° the supernatant fractions were filtered through glass wool and the filtrates were fractionated and analyzed as described under "Experimental Procedure." The vertical bars are the standard errors of the mean. EPI, epinephrine.

FIG. 2. The effect of PGE1 on cyclic AMP levels in isolated fat cells. The experimental conditions were as described under "Experimental Procedure." The lines at the top of each block are the standard errors of the mean, and the numbers in parentheses at the bottom of the figure are the number of experimental points in each group. EPI, epinephrine.

RESULTS

Effects of PGE1 on Cyclic AMP Levels in Rat Epididymal Fat Pad in Vitro—Cyclic AMP levels in fat pads incubated with 1 mM caffeine were 390 pmoles per g, and were increased about 8-fold by the addition of 5.5 μM epinephrine (Fig. 1). The addition of 0.56 or 2.8 μM PGE1 to fat pads incubated with epinephrine and caffeine reduced cyclic AMP levels to 900 and 1380 pmoles, respectively. Thus PGE1 was a potent antagonist of the actions of epinephrine on cyclic AMP accumulation. However, cyclic AMP levels were increased in fat pads incubated with 1 mM caffeine and PGE1, and this change in cyclic AMP levels was large enough to have maximally activated lipolysis (5), although Steinberg et al. (4) reported that PGE1 was without lipolytic activity. When intracellular cyclic AMP levels and glycerol release were compared in fat pads exposed to PGE1 or epinephrine, an apparent dissociation between cyclic AMP levels and lipolysis occurred (Table I). Although both parameters were stimulated by epinephrine, PGE1 (which affected cyclic AMP more strongly than the catecholamine) was without effect on glycerol release.

Effect of PGE1 on Cyclic AMP Levels in Isolated Fat Cells—One of several possible explanations for these apparently paradoxical

TABLE I

Comparison of effects of PGE1 and epinephrine on glycerol release and cyclic AMP levels in fat pads

<p>| Conditions were as described under &quot;Experimental Procedure.&quot; |</p>
<table>
<thead>
<tr>
<th>Additions</th>
<th>Glycerol released</th>
<th>Cyclic AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>μmoles/g, wt</td>
<td>pmol/g, wt + S.E.</td>
</tr>
<tr>
<td>Control (6)</td>
<td>0.21 ± 0.03</td>
<td>120 ± 10</td>
</tr>
<tr>
<td>Control (6) + 55 μM epinephrine</td>
<td>1.47 ± 0.17</td>
<td>480 ± 50</td>
</tr>
<tr>
<td>2.8 μM PGE1 (6)</td>
<td>0.20 ± 0.01</td>
<td>1280 ± 250</td>
</tr>
</tbody>
</table>

aThe numbers in parentheses are the numbers of experiments.
The effects of PGE₇ on whole fat pads was that the prostaglandin was antagonizing the action of epinephrine on the fat cell and stimulating accumulation of cyclic AMP in another type of cell found in the fat pad. Therefore, experiments with isolated fat cells were carried out (Fig. 2). While the addition of 5.5 μM epinephrine caused a large increase in the levels of cyclic AMP, 2.8 μM PGE₇ was without stimulatory activity. Although this concentration of PGE₇ did not increase cyclic AMP levels, the response of the fat cells to epinephrine was almost completely antagonized. This suggested that PGE₇ was stimulating cyclic AMP accumulation in a cell type other than the fat cell. However, it was also possible that the stimulatory effect of PGE₇ was on the fat cell, and that the ability of the cell to respond to PGE₇ with increased cyclic AMP levels had been destroyed by the collagenase treatment used in the preparation of the isolated fat cells. Therefore, experiments designed to clarify this point were undertaken (Fig. 3). Fat pads were incubated at 37°C for about 1 hour with or without collagenase. When the fat pads incubated with collagenase were disintegrated, half of the collagenase-treated samples were filtered through a fiber glass screen and centrifuged, and the fat cells were isolated. The other collagenase-treated samples and the fat pads incubated without collagenase were diluted but not filtered or centrifuged. Caffeine, PGE₁, and epinephrine were then added to the samples as indicated and incubated for 10 min, and cyclic AMP levels were measured. PGE₁ did not stimulate the isolated fat cells, although cyclic AMP levels increased 3-fold in the whole fat pad and in the samples treated with collagenase but not separated. Epinephrine was stimulatory in all three preparations and PGE₁ antagonized the effects of epinephrine.

**Comparison of Activity of PGE₁ with Other Prostaglandins**—The cyclic AMP response of isolated fat cells incubated with 1 mM caffeine and 5.5 μM epinephrine was sensitive to low concentrations of PGE₁ (Fig. 4). The action of epinephrine was inhibited 50% by approximately 0.004 μM PGE₁, and small but significant effects of PGE₁ were detectable at 0.00004 μM, which was the lowest concentration tested. PGF₁α was much less effective than PGE₁, and PGF₁β was without effect on the cyclic AMP response of isolated fat cells.

**Time Course of Action of PGE₁**—PGE₁ lowered intracellular cyclic AMP levels in isolated fat cells very rapidly (Fig. 5). Isolated fat cells were incubated with 1 mM caffeine and 5.5 μM epinephrine for 10 min and then incubated with PGE₁ for 1, 2, 4, or 8 min. During the 1st min of incubation with PGE₁, cyclic AMP levels were not significantly changed, but from Minute 1 to Minute 2 the levels were sharply decreased. A rapid rate of decline in the level of cyclic AMP was maintained between Minutes 2 and 4 and, although it slowed, by Minute 8 cyclic AMP was near the control level.

**Effect of PGE₁ on Cyclic AMP Levels in Isolated Fat Cells Exposed to Epinephrine, Adrenocorticotropic Hormone, Glucagon, and Caffeine**—The stimulatory activity of epinephrine was much less effective than PGE₁. The addition of 2.8 μM PGE₁ (the highest concentration used in these experiments) to isolated fat cells incubated with caffeine and epinephrine resulted in a cyclic AMP concentration of 1000 ± 100 pmol per g. The numbers in parentheses are the numbers of experiments.

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**Fig. 3.** The effects of PGE₁ on cyclic AMP levels in intact fat pads, isolated fat cells, and fat pads treated with collagenase but not separated. Carefully pooled fat pads were incubated at 37°C for about 1 hour with or without collagenase. Half of the collagenase-treated samples were filtered through a fiber glass screen and centrifuged, and the fat cells were isolated. The other collagenase-treated samples and the fat pads incubated without collagenase were diluted but not filtered or centrifuged. Control solution, caffeine, PGE₁ and epinephrine were then added to the samples as indicated, and they were then incubated for an additional 10 min and cyclic AMP levels were measured as described under "Experimental Procedure." The weights used in calculation were the wet weights of the tissues from which the preparations were derived. I.F.C., isolated fat cells; CONT, control; EPI, epinephrine.
or Thyroid-stimulating Hormone in Presence of 1 mM Caffeine—
PGE₁ effectively lowered increased levels of cyclic AMP engendered by adrenocorticotropic hormone, glucagon, and thyroid-stimulating hormone as well as epinephrine (Fig. 6). PGE₁ was at least as effective against adrenocorticotropic hormone, glucagon, and thyroid-stimulating hormone (p < 0.01) as it was against epinephrine.

**Effects of PGE₁ on Other Tissues**—Incubation of rat lung pieces with 2.8 μM PGE₁ in the presence of 1 mM caffeine caused increased levels of cyclic AMP (Table II). PGE₁ did not cause any obvious lowering of cyclic AMP levels when added to lung pieces incubated with epinephrine and caffeine. Cyclic AMP levels were increased in rat hemidiaphragms incubated with PGE₁ and also in spleen slices. Incubation of diaphragms with the combination of PGE₁ and epinephrine led to cyclic AMP levels higher than either alone but not as great as the sum of the two effects, while in spleen there appeared to be a mild inhibition of the effect of PGE₁ by epinephrine. The reason for the high control levels of cyclic AMP in spleen is unknown. PGE₁ also stimulated cyclic AMP accumulation in rat kidney slices and in minced rat testes.

**DISCUSSION**

That cyclic AMP is involved in the antilipolytic action of the prostaglandins on fat cells seems very probable. The role of cyclic AMP in the actions of lipolytic hormones and the methyl xanthines appears to be well established (5), and the

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**TABLE II**

*Effect of PGE₁ on cyclic AMP levels in rat lung, diaphragm, and spleen preparations*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cyclic AMP</th>
<th>pmoles/g, wet wt, ±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung (5)</td>
<td>1 mM caffeine + control</td>
<td>1,100 ± 110</td>
</tr>
<tr>
<td></td>
<td>1 mM caffeine + 2.8 μM PGE₁</td>
<td>2,190 ± 250</td>
</tr>
<tr>
<td></td>
<td>1 mM caffeine + 2.8 μM PGE₁ + 5.5 μM epinephrine</td>
<td>4,230 ± 830</td>
</tr>
<tr>
<td></td>
<td>1 mM caffeine + 2.8 μM PGE₁ + 5.5 μM epinephrine</td>
<td>4,690 ± 640</td>
</tr>
</tbody>
</table>

Diaphragm (5)……. | 260 ± 66 | 850 ± 180 | 1,020 ± 290 | 1,340 ± 240 |
Spleen (6)…….. | 3,710 ± 700 | 13,170 ± 3,960 | 4,100 ± 460 | 4,350 ± 1,650 |
prostaglandins decreased cyclic AMP levels qualitatively, quantitatively, and temporally as they antagonized lipolysis.

However, there are other aspects of the relationships between prostaglandins and cyclic AMP which are much less clear. First is the question of how PGE$_1$, for example, decreases cyclic AMP levels in isolated fat cells. Actions of PGE$_1$ have not been elicited in broken cell preparations of isolated fat cells. The turnover of cyclic AMP in intact cells is high (e.g. see Fig. 5) and it is difficult if not impossible to obtain an unequivocal answer about the site of PGE$_1$ action (i.e. inhibition of adenyl cyclase or stimulation of the phosphodiesterase) in such preparations. Steinberg and Vaughan reported that PGE$_1$ did not antagonize the lipolytic actions of exogenous cyclic AMP or N$^\omega$-2'-O-dibutyryl cyclic AMP (11, 12). This indicated that PGE$_1$ acted to inhibit adenyl cyclase in adipose tissue, as had been suggested earlier by Orloff, Handler, and Bergström (7) working with toad bladder. On the other hand, Mühlbachova, Solyom, and Puglisi (13) have suggested on the basis of lipolytic measurements that prostaglandins stimulate the phosphodiesterase. Such experiments, however, are rather indirect and a final judgment must await the demonstration of an action of PGE$_1$ on one or another of these enzymes. Also, it is possible that the effects of PGE$_1$ on cyclic AMP levels in fat cells may be secondary to another action, e.g. a configurational change in the plasma membrane, or perhaps actions on some as yet undefined second messenger which produces the effect on adenyl cyclase or phosphodiesterase.

Second, the mechanisms by which PGE$_1$ both stimulates cyclic AMP accumulation by itself and antagonizes lipolytic hormones in the whole fat pad are not wholly understood. Since this stimulatory action is lost when fat cells are separated from the rest of the fat pad it seems that another cell type is involved. Unfortunately, this cell type has not been identified. This dualistic action of PGE$_1$ on the fat pad emphasizes the often overlooked fact that tissues in general are heterogeneous and, since cyclic AMP is common to almost all cells, caution must be exercised in drawing conclusions based on whole tissue measurements. PGE$_1$ also stimulated cyclic AMP accumulation in lung, spleen, diaphragm, and other tissues, suggesting that PGE$_1$ either acts on a variety of cell types found in several tissues or on a single cell type common to all of these tissues (e.g. blood vessels, nerves, etc.). It should be noted, however, that PGE$_1$ has been shown to increase cyclic AMP accumulation in two relatively homogeneous systems, broken cell preparations of human leukocytes and platelets.

Finally, it seems possible that there may be a more or less generalized relationship between cyclic AMP and the prostaglandins. Both systems are ubiquitous (1-3, 14) and, in the tissues investigated thus far, cyclic AMP levels are influenced by prostaglandins. In addition, Ramwell and Shaw have shown that certain tissues, including fat, release increased amounts of prostaglandins to the medium when stimulated by hormones (15). This suggests that in some way the release of prostaglandins and the activation of the cyclic AMP mechanism (e.g. adenyl cyclase) may be related. However, it is also quite possible that the release of prostaglandins may be due to an action of increased cyclic AMP, and that prostaglandins may then serve as a feedback mechanism, modulating adenyl cyclase, as has been suggested by Bergström (3).

Acknowledgments—We would like to thank Dr. Earl W. Sutherland for his continued interest in this work. Dr. John E. Pike of The Upjohn Company, Kalamazoo, Michigan, has provided several helpful discussions and the prostaglandins used in these studies, for which we are very grateful. In addition, we would like to acknowledge the valuable technical assistance of Alta Jackson, Nina Wendt, Joseph Campbell, and Ted D. Chrisman.

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