Effects of Adenosine Deaminase on Cyclic Adenosine Monophosphate Accumulation, Lipolysis, and Glucose Metabolism of Fat Cells*

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SUMMARY

In fat cells isolated from the parametrial adipose tissue of rats, the addition of purified adenosine deaminase increased lipolysis and cyclic adenosine 3':5'-monophosphate (cyclic AMP) accumulation. Adenosine deaminase markedly potentiated cyclic AMP accumulation due to norepinephrine. The increase in cyclic AMP due to adenosine deaminase was as rapid as that of theophylline with near maximal effects seen after only a 20-sr incubation. The increases in cyclic AMP due to crystalline adenosine deaminase from intestinal mucosa were seen at concentrations as low as 0.05 μg per ml. Further purification of the crystalline enzyme preparation by Sephadex G-100 chromatography increased both adenosine deaminase activity and cyclic AMP accumulation by fat cells. The effects of adenosine deaminase on fat cell metabolism were reversed by the addition of low concentrations of N6-(phenylisopropyl)adenosine, an analog of adenosine which is not deaminated. The effects of adenosine deaminase on cyclic AMP accumulation were blocked by coformycin which is a potent inhibitor of the enzyme. These findings suggest that deamination of adenosine is responsible for the observed effects of adenosine deaminase preparations.

Protein kinase activity of fat cell homogenates was unaffected by adenosine or N6-(phenylisopropyl)adenosine. Norepinephrine-activated adenylyl cyclase activity of fat cell ghosts was not inhibited by N6-(phenylisopropyl)adenosine. Adenosine deaminase did not alter basal or norepinephrine-activated adenylyl cyclase activity. Cyclic AMP phosphodiesterase activity of fat cell ghosts was also unaffected by adenosine deaminase.

Basal and insulin-stimulated glucose oxidation were little affected by adenosine deaminase. However, the addition of adenosine deaminase to fat cells incubated with 1.5 μM norepinephrine abolished the antilipolytic action of insulin and markedly reduced the increase in glucose oxidation due to insulin. These effects were reversed by N6-(phenylisopropyl)adenosine. Phenylisopropyl adenosine did not affect insulin action during a 1-hour incubation. If fat cells were incubated for 2 hours with phenylisopropyl adenosine prior to the addition of insulin for 1 hour there was a marked potentiation of insulin action. The potentiation of insulin action by prior incubation with phenylisopropyl adenosine was not unique as prostaglandin E1, and nicotinic acid had similar effects. The present results lend further support to the hypothesis that adenosine is an important modulator of hormone action on fat cells.

Adenosine inhibits cyclic AMP* accumulation in rat fat cells (1–5). Adenosine is continuously released to the medium during incubation of fat cells (3). If this is the case, then the removal of adenosine by adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) should increase cyclic AMP accumulation. Fain (2) found that 0.5 μg per ml of crystalline adenosine deaminase isolated from calf intestinal mucosa increased cyclic AMP and lipolysis. Schwabe and L bert (6) have confirmed this observation.

The present studies are a detailed investigation into the effects of adenosine deaminase on fat cell metabolism. The effects of N6(phenylisopropyl)adenosine were also examined as this adenosine analog is not deaminated by the enzyme and can be used to reverse the effects of the adenosine deaminase.

METHODS

Free white fat cells were obtained from 120- to 160-g female Sprague-Dawley rats (Charles River CD strain) fed laboratory chow ad libitum. White fat cells were isolated by a modification of the procedure of Rodbell (7) from the pooled parametrial adipose tissue of three or more rats. Krebs-Ringer phosphate buffer of the following composition was used in all experiments: NaCl, 128 mM; CaCl2, 1.4 mM; MgSO4, 1.4 mM; KCl, 5.2 mM; and NaH2PO4, 10 mM. The buffer was prepared daily and adjusted to pH 7.4 with NaOH after the addition of Armour bovine Fraction V albumin powder (No. 43405). All incubations were done in duplicate for each experiment at 37°C in a shaking incubator in the absence of glucose unless otherwise stated with a final volume of 1 ml.

In all of the studies except those in Fig. 1, cyclic AMP accumulation was measured in cells plus medium after adding 0.1 ml of 2 N HCl to the tubes (1 ml of buffer + cells) prior to placing in a boiling water bath for 1 min. The tubes were then allowed to cool and 0.05 ml of 4 N NaOH was added. The tubes were mixed

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and centrifuged prior to the removal of 20-μl aliquots for the determination of cyclic AMP. In each experiment, no more than 50 mg of fat cells were incubated per ml of medium which meant that the 20-μl aliquot taken for cyclic AMP analysis represented less than 1 mg of fat cells. Cyclic AMP was determined by a modification (1, 2) of the protein kinase binding assay of Gilman (8).

In the studies shown in Table 1, cyclic AMP release to the medium was analyzed by taking 20-μl aliquots of the medium at the end of the incubation and prior to the addition of acid. In the same experiments, cyclic AMP was also measured after barium-zinc treatment and column chromatography. Membrane-bound adenylate cyclase and cyclic AMP phosphodiesterase activity were determined on ghosts prepared by hypotonic lysis of fat cells (9). The ghosts (50 μg of protein) were used immediately and incubated for 10 min at 37°C in a total volume of 100 μl containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl2, 30 mM KCl, 8 mM phosphoenolpyruvate, 10 μg of pyruvate kinase, and 1 mM ATP. If 5 μM theophylline was added to the incubation mixture, all of the values were slightly higher and the degradation of added cyclic AMP at a concentration of 0.5 μM was blocked. Cyclic AMP was determined after the reaction mixture containing ghosts was boiled for 3 min and then diluted to a volume of 2 ml. Aliquots (20 μl) were measured by the protein kinase binding assay for cyclic AMP. However, the cyclic AMP binding protein was that from the 10,000 × g supernatant of homogenized bovine adrenal glands and the assay was conducted as described by Brown et al. (10) to eliminate interference by ATP.

Cyclic AMP phosphodiesterase activity of fat cell ghosts was measured under exactly the same conditions used for the adenylate cyclase assay in the presence of 0.4 μM cyclic AMP added at the start of the incubation. The cyclic AMP was measured by the protein kinase procedure (10).

Glycerol release and fatty acid release were analyzed as described previously (11). In each experiment, the values for lipolysis and cyclic AMP were based on the mean of duplicate tubes. The data are expressed per g of fat cells calculated from the amount of triglyceride present per tube (7). Glucose oxidation was measured as described by Rodbell (7) and ATP by the procedure of Fain and Rosenthal (12). Protein kinase activity was determined as previously described except that γ-labeled [32P]ATP was used as substrate (13). White fat cells were homogenized with three strokes of a glass homogenizer fitted with a Teflon pestle (1800 rpm) in a solution containing 0.15 M KCl. After

![Fig. 1. Time course of cyclic AMP accumulation due to adenosine deaminase. Fat cells (28 mg per tube) were incubated for 20 to 600 s in the absence (left) or presence of 0.5 μg per ml of adenosine deaminase (right). Norepinephrine (NOREPI) (A) was present at a concentration of 0.15 μM, theophylline (THEO) (B) at 100 μM, the combination of norepinephrine plus theophylline (NE + THEO) are shown by stars and basal values by open circles.](http://www.jbc.org/)

**TABLE I**

Comparison of cyclic AMP and glycerol release

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<th>Additions</th>
<th>Glyceral Release</th>
<th>Cyclic AMP</th>
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<th>Total Cyclic AMP</th>
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<tr>
<td></td>
<td>(μmoles/g)</td>
<td>(μmoles/g)</td>
<td>(μmoles/g)</td>
<td>(μmoles/g)</td>
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<tr>
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<td>2 min. 10 min.</td>
<td>2 min. 10 min.</td>
<td>2 min. 10 min.</td>
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<tr>
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<td>0.8</td>
<td>0.08</td>
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<td>0.1</td>
<td>2.5</td>
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<td>0.05</td>
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<td>0.05</td>
<td>0.08</td>
</tr>
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<td>6.5</td>
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<td>5.3</td>
<td>0.07</td>
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<td>5.1</td>
<td>0.47</td>
<td>0.59</td>
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<td>4.2</td>
<td>0.04</td>
<td>0.30</td>
</tr>
<tr>
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<td>0.3</td>
<td>6.2</td>
<td>0.43</td>
<td>1.20</td>
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centrifugation at $12,000 \times g$ for 10 min at 4°, 10-µl aliquots, containing 60 µg of protein, from the soluble fraction were assayed for protein kinase activity in the absence and presence of cyclic AMP. The assay mixture contained 1 µmol of potassium phosphate (pH 6.5), 0.36 µmol of magnesium acetate, 0.12 µmol of $[\gamma^{32}P]ATP$, and 3 µg per ml of histone in a final volume of 0.1 ml.

Crystalline adenosine deaminase preparations from calf intestinal mucosa were obtained from either Sigma or Boehringer Mannheim. These preparations were able to deaminate about 500 to 600 pmol of adenosine per mg of protein in 1 min at 37° if the adenosine concentration was 100 µM. The purified adenosine deaminase used in Figs. 2 and 3 was prepared by Sephadex G-100 chromatography of the commercial preparations. Adenosine deaminase activity was determined spectrophotometrically by the decrease in absorbance at 260 nm (14). Dr. Harold Stork of Boehringer Mannheim supplied the $N^\alpha$-(phenylisopropyl)adenosine and Dr. R. Agarwal and R. P. Parks the coformycin, which was a gift from Dr. H. Umezawa of the Institute of Microbial Chemistry Shinagawa-ku, Tokyo, Japan to Dr. A. W. Sonfett.

RESULTS

The addition of 0.5 µg per ml of adenosine deaminase to the medium markedly increased cyclic AMP accumulation by white fat cells in the absence or presence of norepinephrine, theophylline, or both agents within 20 s, which was the earliest time period examined (Fig. 1). The increase in cyclic AMP of cells plus medium due to theophylline was also equally rapid in the onset with near maximal stimulation noted at 20 s. There was no detectable rise in cyclic AMP due to 0.15 µM norepinephrine except in the presence of theophylline or adenosine deaminase. However, norepinephrine alone was able to increase lipolysis at 10 min (Table I).

The addition of 0.5 µg per ml of norepinephrine increased cyclic AMP accumulation to 10 nmol per g (Table I). This was a 10-fold or greater increase in cyclic AMP than was seen with 0.15 µM norepinephrine in the presence of theophylline and equivalent to that noted with the combination of adenosine deaminase, 0.15 µM norepinephrine, and theophylline. Apparently, if enough catecholamine is present, a maximal increase in cyclic AMP can be seen with 100 µM theophylline in the absence of adenosine deaminase.

Table I also demonstrates that the analysis of cyclic AMP content in unpurified extracts gave values similar to those seen after the purification of the cyclic AMP by column chromatography and barium-zinc treatment. We have observed similar effects of adenosine deaminase, norepinephrine, and theophylline on the accumulation of labeled cyclic AMP in cells previously exposed to tritiated adenosine. 2

The possibility that the effects of adenosine deaminase were due to impurities in the crystalline preparations which are available commercially was considered. Adenosine deaminase was purified by chromatography on Sephadex G-100. Comparison of the crystalline and purified adenosine deaminase by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and mercaptoethanol by the procedure of Weber and Osborn (15) indicated the presence of one main band and several smaller bands in the crystalline material which were virtually absent in the purified preparations (data not shown). The specific activity for the main peak of adenosine deaminase obtained after Sephadex G-100 chromatography was about 20% greater than that of the starting material as was its ability to stimulate cyclic AMP accumulation during a 5-min incubation with norepinephrine (data not shown). As little as 0.01 µg per ml of the adenosine deaminase was able to increase both lipolysis and cyclic AMP whereas maximal stimulation of both parameters was seen with 0.5 µg per ml and increasing deaminase concentration to 2 µg per ml gave no further effect (data not shown).

The similarity in effects of the purified adenosine deaminase

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2 John N. Fain and Paul R. Wieser, unpublished experiments.
with those of relatively impure commercial preparations suggested that adenosine deaminase was responsible for the effects noted. If the enzyme was boiled for 5 min prior to use there was no stimulatory effect on lipolysis.\textsuperscript{2} We have tested the commercial preparations of adenosine deaminase and found no detectable deamination of ATP, cyclic adenosine monophosphate, or other nucleotides. The enzyme will deaminate 2'-deoxyadenosine as reported previously (16). The activity of the deaminase was not inhibited by substances present in the albumin buffer or released by fat cells.

In the experiments shown in Fig. 2, the effects of adenosine deaminase, purified by chromatography on Sephadex G-100, in the presence of varying concentrations of norepinephrine either without or with theophylline were examined. In these experiments purified adenosine deaminase alone increased both cyclic AMP and lipolysis whereas 100 $\mu M$ theophylline had an equivalent lipolytic effect but produced little detectable rise in cyclic AMP. No increase in cyclic AMP was seen with 0.075 or 0.15 $\mu M$ norepinephrine despite their near maximal stimulation of lipolysis. However, in the presence of adenosine deaminase, a clearly observable rise in cyclic AMP due to either concentration of norepinephrine was seen (Fig. 2).

Adenosine deaminase addition to the medium in which fat cells were incubated did not affect ATP content. In three experiments, the ATP content after a 60-min incubation was 101 nmol per g in the absence and 95 in the presence of 0.15 $\mu M$ norepinephrine. In the presence of 0.5 $\mu g$ per ml of adenosine deaminase, the value for ATP was 96 in the absence and 99 in the presence of norepinephrine.

The nucleoside antibiotic coformycin is a potent inhibitor of adenosine deaminase (17). We confirmed this as crystalline calf intestinal adenosine deaminase (0.1 $\mu g$ per ml or 3 nM) deaminated about 50 nmol per min of adenosine at a substrate concentration of 75 $\mu M$ in the absence and less than 0.1 nmol per min in the presence of 100 $\mu M$ coformycin.

Coformycin (100 $\mu M$) reversed the marked increase by adenosine deaminase of cyclic AMP accumulation due to norepinephrine and theophylline (Fig. 3). Coformycin also potentiated the inhibition by 0.1 and 1 $\mu M$ adenosine of cyclic AMP accumulation (Fig. 3). In another experiment, glycerol release due to 0.1 $\mu g$ per ml of adenosine deaminase (3 nM) was 4 $\mu mol$ per g over 10 min in the absence and less than 0.1 $\mu mol$ per g in the presence of 3.5 $\mu M$ coformycin.

The amount of coformycin required to inhibit cyclic AMP accumulation due to norepinephrine and theophylline was very low (Fig. 4). As little as 0.3 $\mu M$ coformycin reduced cyclic AMP in the presence of adenosine. Coformycin (15 $\mu M$) completely blocked the effects of 3 $\mu M$ adenosine deaminase (Fig. 4). Coformycin appears to be an extremely potent inhibitor of adenosine deaminase effects on fat cell metabolism. Apparently only a slight molar excess of coformycin over adenosine deaminase is sufficient to block all effects of the enzyme. Furthermore, the inhibition by coformycin of the adenosine deaminase was not reversed by the addition of large amounts of adenosine.

If the effects of adenosine deaminase are solely due to the removal of adenosine, then the addition of an analog of adenosine which is not deaminated but is able to mimic the effects of adenosine should result in a reversal of deaminase action. The lipolytic action of 0.2 $\mu g$ per ml of adenosine deaminase was markedly inhibited by only 0.06 $\mu M$ N<sub>H</sub>-phenylisopropyl)adenosine (Fig. 5). The increased lipolysis due to theophylline or 1-methyl-3-isobutylxanthine was unaffected by phenylisopropyl adenosine (Fig. 5). However, the increase in cyclic AMP accumulation was 3.2 $\mu mol$ per g over 10 min in the absence and 0.7 in the presence of 2 $\mu M$ adenosine in three experiments. In the presence of 2 $\mu M$ inosine, 2 $\mu M$ NH<sub>4</sub>Cl, or both, cyclic AMP accumulation was 3.5 $\mu mol$ per g.

![Fig. 4. Amount of coformycin required to inhibit cyclic AMP accumulation. Fat cells (30 mg per tube) were incubated for 10 min in medium containing 1 $\mu M$ adenosine, 1.5 $\mu M$ norepinephrine, and 100 $\mu M$ theophylline either without (A) or with (●) adenosine deaminase (0.1 $\mu g$ per ml or 3 nM).](http://www.jbc.org/)

![Fig. 5. N<sub>H</sub>-phenylisopropyl)adenosine versus methylxanthines. Fat cells (29 mg per tube) were incubated in the presence of 0.2 $\mu g$ per ml of adenosine deaminase for 2 min (cyclic AMP) or 30 min (glycerol release). Theophylline (O), theophylline and 0.06 $\mu M$ PIA which stands for N<sub>H</sub>-phenylisopropyl)adenosine (●), MIX which represents 1-methyl-3-isobutylxanthine (†), and MIX plus PIA (A) are the means of duplicate tubes from a single experiment. The basal value for cyclic AMP was unaffected by N<sub>H</sub>-phenylisopropyl)adenosine (PIA). Cyclic AMP was measured in cells plus medium.](http://www.jbc.org/)
The possibility that adenosine might affect protein kinase activity of fat cells was examined with negative results. The basal protein kinase activity of the $2,000 \times g$ supernatant from fat cells was 3.3 nmol of $^3H$ incorporated per mg of protein in the absence, 3.5 in the presence of 0.2 $\mu$M adenosine, and 3.7 with phenylisopropyl adenosine. There was also no significant effect of 0.2 $\mu$M adenosine or $N^6$(phenylisopropyl)adenosine on the increase in protein kinase activity seen in the presence of 1, 2, or 20 $\mu$M cyclic AMP.

Basal adenylate cyclase activity of fat cell ghosts was increased from 0.12 nmol of cyclic AMP formed per mg of protein over 10 min to 0.18 in the presence of 0.5 $\mu$g per ml of adenosine deaminase for seven paired experiments. Norepinephrine (200 $\mu$M) increased cyclic AMP accumulation to 1.2 nmol per mg of protein and in the presence of adenosine deaminase the values were only 12 $\pm$ 7% higher in seven paired experiments. This was a nonsignificant increase in the response to norepinephrine. In the presence of 40 $\mu$M norepinephrine, adenylate cyclase activity over 10 min was 0.45 nmol of cyclic AMP formed per mg of protein and was increased by only 14 $\pm$ 7% in the presence of 0.5 $\mu$g per ml of adenosine deaminase in seven experiments.

In the presence of 5 mM theophylline, adenylate cyclase activity was increased to 0.26 nmol of cyclic AMP whereas the response to 0.2 $\mu$M norepinephrine was increased by only 30% in two experiments. We also confirmed our previous finding that 5 $\mu$M 2',5'-dideoxyadenosine inhibited norepinephrine-activated cyclase by 50%; whereas the same concentration of $N^6$(phenylisopropyl)adenosine had no effect on adenylate cyclase (1). The amount of cyclic AMP degradation at an initial concentration of 0.4 $\mu$g per ml by the fat cell ghosts used in these experiments was about 50% to 80% over 10 min and unaffected by adenosine deaminase (data not shown). Cyclic AMP phosphodiesterase was measured under conditions exactly the same as those for the adenylate cyclase assay.

The effects of phenylisopropyl adenosine and adenosine deaminase on glucose metabolism were investigated in the studies shown in Tables II and III and Fig. 6. The addition of adenosine deaminase to cells incubated with 1.5 $\mu$M norepinephrine did not further increase lipolysis over a 1-hour period but did abolish the antilipolytic action of insulin and markedly reduced the stimulation of glucose metabolism by insulin, particularly at 50 micro-units per ml (Table II). These effects of adenosine deaminase were completely reversed by 0.1 $\mu$M phenylisopropyl adenosine. There was little effect of adenosine deaminase or phenylisopropyl adenosine on glucose metabolism in the absence of norepinephrine during a 1-hour incubation.

The effects of adenosine deaminase and theophylline were similar in that both agents abolished the antilipolytic action of insulin on norepinephrine-activated lipolysis and reduced insulin-stimulated glucose metabolism. However, phenylisopropyl adenosine was unable to restore the antilipolytic action of insulin in the presence of theophylline (Table II). $N^6$(phenylisopropyl)adenosine alone increased slightly basal glucose metabolism in fat cells but did not affect insulin-stimulated oxidation over a 1-hour incubation (Fig. 6).

If fat cells were incubated with 0.1 $\mu$M $N^6$(phenylisopropyl)-adenosine for 2 hours prior to the addition of insulin, there was a marked increase in the ability of low concentrations of insulin (20 to 100 micro-units per ml) to increase glucose oxidation (Table III). There was little effect of the adenosine analog alone on glucose oxidation. Glycerol release was increased from 9 to 24 $\mu$mol per g in the presence of 0.1 $\mu$g per ml of adenosine deaminase but $N^6$(phenylisopropyl)adenosine completely blocked lipolysis even in the presence of adenosine deaminase. Insulin addition

<table>
<thead>
<tr>
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<tr>
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<td><strong>Glucose release, $\mu$moles/g fat cells</strong></td>
<td><strong>Glucose release, $\mu$moles/g fat cells</strong></td>
</tr>
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</tr>
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</table>

**TABLE II**

**Effect of adenosine deaminase and phenylisopropyl adenosine on stimulation of [1-14C]glucose oxidation by insulin and on antilipolytic effect of insulin**

White fat cells (30 mg per tube) were incubated for 1 hour in the presence of 0.5 mM [1-14C]glucose with 50 or 200 micro-units per ml of insulin, adenosine deaminase (0.5 $\mu$g per ml), $N^6$(phenylisopropyl)adenosine (0.1 $\mu$g), norepinephrine (1.5 $\mu$g), or theophylline (50 $\mu$g) which were present during the entire incubation period. The effect of insulin is shown as the increase (+) or decrease (-) $\pm$ standard error due to insulin for three paired experiments.
TABLE III

Effect of \(N^6\)-(phenylisopropyl)adenosine, prostaglandin \(E_1\), and nicotinic acid on insulin-stimulated glucose oxidation

Fat cells (30 mg per tube) were incubated for 3 hours in the presence of 0.5 mM \([1-^{14}C]glucose\) and in the presence or absence of 0.1 \(\mu M\) \(N^6\)-(phenylisopropyl)adenosine (PIA), 0.2 \(\mu g\) per ml of prostaglandin \(E_1\) (PGE), or 1 \(\mu g\) per ml of nicotinic acid. Adenosine deaminase (0.1 \(\mu g\) per ml) was present where indicated. Insulin was added after 2 hours and the incubation continued for an additional hour. CO\(_2\) was collected for the last hour. Glycerol release was 9 \(\mu mol\) per g in the absence and 24 \(\mu mol\) per g in the presence of adenosine deaminase. These values were unaffected by the presence of insulin at any concentration. In the presence of PIA, PGE, or nicotinic acid the glycerol release was less than 1 \(\mu mol\) per g even in the presence of adenosine deaminase.

![Diagram](insert)

![Graph](insert)

**FIG. 6.** Dose response to \(N^6\)-(phenylisopropyl)adenosine in the presence and absence of insulin. Fat cells (30 mg per tube) were incubated for either 1 hour (left panel) or 3 hours (right panel) in the presence of 0.5 mM \([1-^{14}C]glucose\) and the concentration of \(N^6\)-(phenylisopropyl)adenosine as indicated. Insulin (20 micro-units per ml) (●) was present from the start (no preincubation) or added after 2 hours (2-hour preincubation) and the incubation continued for an additional hour. In both cases, CO\(_2\) was collected for the last hour. The data are the results of three experiments.

during the last hour of the 3-hour incubation did not affect basal lipolysis or that due to adenosine deaminase.

In fat cells incubated for 2 hours prior to the addition of 20 micro-units per ml of insulin, the presence of 0.01 \(\mu M\) \(N^6\)-(phenylisopropyl)adenosine markedly increased insulin-mediated glucose oxidation (Fig 6). Increasing the concentration of \(N^6\)-(phenylisopropyl)adenosine to 1 \(\mu M\) did not give any further increase in insulin-stimulated glucose metabolism.

The question arises as to whether other inhibitors of cyclic AMP accumulation and lipolysis such as nicotinic acid or prostaglandin \(E_1\) would mimic the action of phenylisopropyl adenosine. The data in Table III show that prior incubation for 2 hours of fat cells with all three agents markedly enhanced the stimulation by insulin of glucose oxidation during the next hour.

DISCUSSION

The finding that adenosine deaminase activated lipolysis and markedly potentiated the increase in cyclic AMP accumulation due to norepinephrine supports the hypothesis that adenosine is an important regulator of fat cell metabolism (1–4). The possibility that impurities in the adenosine deaminase preparations are responsible for the observed effects is unlikely. Further purification of the commercial preparations of the enzyme by Sephadex G-100 chromatography markedly reduced the contaminants while increasing the ability of the enzyme to affect fat cell metabolism. The effects of adenosine deaminase could be reversed by the presence of small amounts of \(N^6\)-(phenylisopropyl)adenosine, which is not deaminated yet possesses the full biological activity of adenosine. Furthermore, all of the effects of adenosine deaminase were reversed by coformycin which is a potent inhibitor of the enzyme. These data support
the supposition that preparations of adenosine deaminase increase lipolysis and cyclic AMP accumulation by removing adenosine. Schwabe and Ebert (6) have also found that adenosine deaminase preparations from calf intestine increased both lipolysis and cyclic AMP accumulation by fat cells.

Low concentrations of norepinephrine stimulated lipolysis but did not result in measurable increases in cyclic AMP or at best nonsignificant differences from basal levels (Fig. 1). One explanation for this observation is that there is little relationship between cyclic AMP and lipolysis. Other possibilities are that small increases in the cyclic AMP level or a change in cyclic AMP compartmentalization are sufficient to activate lipolysis and assays for total cyclic AMP lack sufficient sensitivity to detect these changes. Preliminary results in this laboratory have indicated that there are changes in labeled cyclic AMP binding to cellular proteins in response to doses of norepinephrine that generally produce no measurable increase in cyclic AMP.

The rapidity with which adenosine deaminase and theophylline were able to increase cyclic AMP accumulation is of interest. Possibly, theophylline primarily increases cyclic AMP accumulation by inhibiting the action of adenosine. This is supported by the finding that with low concentrations of fat cells there was a marked increase in cyclic AMP accumulation seen with catecholamines alone which was not further potentiated by methylxanthines (18). Further studies will have to be done to prove or disprove this hypothesis but for the moment it appears as likely that methylxanthines act to inhibit adenosine action on cyclic AMP accumulation of fat cells as that they work by inhibiting cyclic AMP phosphodiesterase. In both the present studies (Fig. 3) and those reported by Schwabe and Ebert (6), low concentrations of theophylline (100 μM or less) accelerated lipolysis but did not elevate cyclic AMP whereas adenosine deaminase increased both parameters. It was easy to see an increase in cyclic AMP due to methylxanthines in the presence of adenosine deaminase (Fig. 5). The inhibitory effect of phenylisopropyl adenosine on increases in cyclic AMP due to theophylline or methylisobutylxanthine were partially overcome by increasing the concentrations of the xanthines (Fig. 5).

There is a marked variability in the literature with respect to the increase in cyclic AMP accumulation by fat cells seen in the presence of catecholamines (19). Butcher et al. (20) originally reported that in isolated white fat cells there was no increase in cyclic AMP due to 1 mM caffeine and only a very small rise with 5.5 mM epinephrine. However, they were able to see large increases in cyclic AMP if both agents were present (20). Some workers have seen large increases in cyclic AMP due to either catecholamines or methylxanthines (18). Possibly these differences are due to the net accumulation of adenosine in the medium. This hypothesis suggests that if there is little adenosine accumulation in the medium when small amounts of cells are incubated per ml or there is high deaminase activity, a marked rise in cyclic AMP due to catecholamines alone will be observed. If there is substantial accumulation of adenosine due to low deaminase activity, a large number of fat cells per ml, or both, then there will be no rise in cyclic AMP due to catecholamines unless methylxanthines are present to antagonize the inhibition of cyclic AMP accumulation by adenosine.

The data of Schwabe et al. (3) indicated that when fairly high concentrations of fat cells are incubated, about 10 μmol of adenosine per g of fat cells appeared in the medium and this was not affected by catecholamines. If there is a constant release of adenosine, then removal of adenosine by either reuptake by the fat cells or deamination to inosine will determine the net level. We suspect that the activity of adenosine deaminase in the medium is a major variable in determining adenosine content of the medium. This may be influenced by factors in albumin, by the contamination of albumin with adenosine deaminase, by the extent to which cells are washed after collagenase digestion, or by other unknown parameters. We have found that after digestion of adipose tissue with collagenase and removal of particulate matter by centrifugation, the infranatant would deaminate 40 nmol of adenosine per ml in 1 min.

The negative effects of adenosine deaminase on adenylyl cyclase activity of fat cell ghosts were expected because neither adenosine nor N6 (phenylisopropyl) adenosine at micromolar concentrations were effective inhibitors of adenylyl cyclase activity in ghosts (1). A reasonable explanation for the present results and those reported previously is that in the process of hypotonic lysis of fat cells to prepare ghosts the sites at which adenosine, insulin, prostaglandin E1, and possibly theophylline inhibit cyclic AMP accumulation are lost. If this were the case, then one would not expect adenosine deaminase to be able to stimulate cyclic AMP accumulation as the ghosts would be relatively insensitive to adenosine. The effects of adenosine nucleosides on adenylyl cyclase appears to be via a quite different mechanism than that involved in inhibition of intracellular cyclic AMP accumulation as the structure activity relationships were quite different for inhibition of cyclase versus cyclic AMP accumulation. We did see inhibition of adenylyl cyclase by adenosine analogs (1) but in those studies 2,5-di(2-deoxyadenosine) was the most potent compound in inhibiting adenylyl cyclase (1) whereas it had less effect on cyclic AMP accumulation in intact cells (2). The reverse was seen with N6 (phenylisopropyl)-adenosine as it was effective in inhibiting intracellular cyclic AMP accumulation but ineffective in inhibiting adenylyl cyclase (1). These results illustrate again the great complexity in extrapolating from studies on isolated enzymes such as adenylyl cyclase to cyclic AMP accumulation in intact cells. There was also little effect of adenosine or the phenylisopropyl adenosine on protein kinase or of adenosine deaminase on cyclic AMP phosphodiesterase activity. Previously we reported that somewhat higher concentrations of adenosine (50 μM) inhibited cyclic AMP phosphodiesterase in the soluble supernatant of fat cells (1). Miyamoto et al. (21) found that high concentrations of adenosine (50 μM or greater) also inhibited brain protein kinase.

The reversal of adenosine deaminase action by coformycin was most interesting as it appeared that 1 mM coformycin would block the effect of 1 nm adenosine deaminase. Under appropriate conditions, coformycin is a remarkable antilipolytic agent. There are few other agents besides insulin which will work at nm concentrations to inhibit lipolysis. Coformycin should be useful in studies measuring the accumulation of adenosine by fat cells because in the presence of coformycin, one could inhibit adenosine removal by deamination.

Removal of adenosine from the medium by adding adenosine deaminase did not reduce the ability of insulin to stimulate glucose metabolism or inhibit lipolysis during short term incubations. There was an inhibition of insulin action by adenosine deaminase in the presence of norepinephrine but this may have been secondary to the marked increase in cyclic AMP accumulation due to adenosine deaminase in the presence of catecholamines. In the presence of a concentration of norepinephrine which maximally stimulated lipolysis, the addition of adenosine deaminase did not result in any further increase in lipolysis but did abolish the anti-
lipolytic action of insulin. This effect was reversed by the phenylisopropyl adenosine. There was also little effect of phenylisopropyl adenosine on basal glucose metabolism or on the ability of insulin to stimulate glucose oxidation except when cells were incubated with the adenosine analog for 2 hours prior to the studies. Under those circumstances, there was a marked increase in the response of the cells to low concentrations of insulin.

The data presented in this manuscript support the hypothesis that adenosine is an agent which primarily decreases cyclic AMP accumulation, whereas insulin acts to inhibit lipolysis via a non-cyclic AMP-dependent mechanism. We have noted previously that under appropriate conditions one can see a marked synergism between the antilipolytic effects of insulin and adenosine (2). Our results and those of Schwabe et al. (5) are all compatible with the suggestion that insulin cannot inhibit lipolysis due to high concentrations of lipolytic agents unless cyclic AMP accumulation is maintained at low levels by adenosine. These data suggest that insulin works by a different mechanism than does adenosine. However, in order for insulin to inhibit lipolysis, the presence of a low concentration of cyclic AMP is required. The marked effect of adenosine on insulin-stimulated glucose oxidation seen in cells incubated for 2 hours with phenylisopropyl adenosine turned out not to be due to any direct interaction between adenosine and insulin as prostaglandin E2 and nicotinic acid mimicked the action of the phenylisopropyl adenosine. The three agents have in common the ability to markedly reduce cyclic AMP accumulation and lipolysis.

Schwabe et al. (5) have reported that adenosine at concentrations as low as 0.001 μM can stimulate glucose oxidation due to 30 micromolars per ml of insulin during a 30 min incubation. However, the amounts of fat cells present in their studies were exceedingly low (1 mg of cells per ml) as compared to 30 to 40 mg of cells per ml used in our studies. Thus, adenosine accumulation in the medium may have been so low that basal levels of both cyclic AMP and lipolysis were elevated. Unfortunately, no data on either of these parameters were presented for cells incubated at a concentration of 1 mg per ml (5). The potentiation by adenosine of insulin action in the studies of Schwabe et al. (5) could be secondary to inhibition of elevated cyclic AMP accumulation. Based on our findings for longer term incubations, other agents such as nicotinic acid or prostaglandin E2 should potentiate insulin action when low concentrations of fat cells are incubated for shorter periods. We have been able to see such potentiation of insulin action by adenosine as well as by 1GE1 and nicotinic acid when 10 mg per ml or less of fat cells were incubated for 1 hour. 3

Sattin and Rall (22) first reported effects of adenosine on cyclic AMP accumulation. In brain slices, adenosine markedly increased cyclic AMP and this effect was blocked by theophylline. This was an unusual finding as theophylline generally potentiates the ability of agents to increase cyclic AMP accumulation. In fat cells, there also appears to be competition between theophylline and adenosine in the opposite direction. Similar results were seen with methylisobutylxanthine which is a more potent methylxanthine than theophylline.

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Effects of adenosine deaminase on cyclic adenosine monophosphate accumulation, lipolysis, and glucose metabolism of fat cells.
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