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Inhibition of Adenosine Cyclic 3′,5′-Monophosphate Accumulation in Fat Cells by Adenosine, N^6-(Phenylisopropyl)adenosine, and Related Compounds

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SUMMARY

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The hypothesis that adenosine may serve as a physiological feedback regulator of adenylate cyclase in rat white fat cells was examined. Adenosine (0.2 μM), when added to incubated fat cells, caused 50% inhibition of the increase in adenosine cyclic 3′,5′-monophosphate accumulation due to 1.5 μM norepinephrine in the presence and absence of methylxanthines. The onset of adenosine inhibition was rapid and, if added 1 min after activators of adenylate cyclase, it reduced cyclic AMP accumulation during the next minute. Adenosine 5′-monophosphate and adenosine 5′-triphosphate were less effective than adenosine as inhibitors of cyclic AMP accumulation. Of a variety of nucleosides only N^6-(phenylisopropyl)adenosine was more effective than adenosine in inhibiting cyclic AMP accumulation. Under conditions in which adenosine markedly reduced the small increase in cyclic AMP accumulation due to norepinephrine alone, it did not reduce lipolysis. In contrast, insulin reduced lipolysis but had a smaller effect on cyclic AMP accumulation. Adenosine inhibited lipolysis in the presence of insulin but produced no greater effect on cyclic AMP accumulation than was seen with adenosine alone. Drugs such as dipyridamole or papaverine did not affect cyclic AMP accumulation due to norepinephrine or block the inhibitory action of adenosine. Inhibition of cyclic AMP accumulation by adenosine was demonstrable also when cells were incubated in calcium-free buffer containing 0.25 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid. The addition of adenosine deaminase to incubated fat cells increased basal lipolysis and cyclic AMP and potentiated the norepinephrine-induced increase in cyclic AMP. The present results suggest that adenosine or related compounds may serve a physiologically important function as feedback regulators of adenylate cyclase.

INTRODUCTION

Adenosine and related nucleosides have been shown to be potent inhibitors of adenylate cyclase activity in fat cell ghosts and of cyclic 3′,5′-AMP accumulation due to catecholamines, in both the absence and presence of methylxanthines (1). Paradoxically, adenosine was much less effective as an antilipolytic agent, since almost complete inhibition of cyclic AMP accumulation due to norepinephrine (1.5 μM) was not accompanied by inhibition of lipolysis (1). These studies suggested that adenosine or similar compounds might function as physiological

regulators of adenylate cyclase. The present experiments were designed to investigate further the effects of adenosine on cyclic AMP accumulation and lipolysis in fat cells.

METHODS

Free white fat cells were obtained from the parametrial adipose tissue of three or more 120–160-g female Sprague-Dawley rats (Charles River CD stain) fed laboratory chow ad libitum (2). Krebs-Ringer-phosphate buffer of the following composition was used in all experiments: NaCl, 128 mM; $CaCl_2$, 1.4 mM; $MgSO_4$, 1.4 mM; KCl, 5.2 mM; Na_2HPO_4, 10 mM; and albumin, 3%. The buffer was prepared daily and adjusted to pH 7.4 with NaOH after addition of Armour bovine Fraction V albumin powder (lot 38,311). All incubations were carried out at 37° in a shaking incubator, in the absence of glucose, in buffer containing 3% albumin.

The adipose tissue (2–3 g) was incubated for 45 min with 8 ml of albumin buffer containing 0.5 mg/ml of crude bacterial collagenase (Worthington). The pooled fat cells were collected, rinsed with buffer, and then incubated for 15 min in 6–8 ml of buffer. The cells were rinsed twice and distributed among the various incubation tubes. At the end of the incubation period 50 μl of the medium were analyzed for glycerol (1), and then 0.1 ml of 2 N HCl was added just prior to placing the tubes in a boiling water bath for 1 min. After removal from the water bath 0.05 ml of 4 N NaOH was added, and the tubes were mixed and then centrifuged prior to removal of duplicate 20-μl aliquots for determination of total cyclic AMP by the procedure of Gilman (3) as described previously (1). No interference by adenosine or phenylisopropyladenosine with the assay for cyclic AMP was detected at the concentrations used in the experiments.

Dipyridamole and papaverine were dissolved in dimethyl sulfoxide, and 10-μl aliquots were added to each incubation tube. In experiments in which these agents were present 10 μl of dimethyl sulfoxide were added to the control tubes. All nucleosides and nucleotides were dissolved in water. Dipyridamole [Persantine, 2,2′,2″,2‴-(4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine-2,6-diyldinitrilo)tetraethanol] was a gift of Geigy Pharmaceuticals, and papaverine (6,7-dimethoxy-1-veratrylisoquinoline) was obtained from Sigma Chemical Company. The sources of the nucleosides were as follows: 2′,5′-dideoxyadenosine, 2-fluoroadenosine, and 9-β-arabinofuranosyladenine, gifts of Drug Research and Development, Chemotherapy, National Cancer Institute; N^6-(phenylisopropyl)adenosine, Dr. Harold Stork, Boehringer-Mannheim; tubercidin (7-deazaadenosine), a gift of the Upjohn Company; puromycin [3′-(α-amino-*p*-methoxyhydrocinnamamido)-3′-deoxy-*N*,*N*-dimethyladenosine) and its aminonucleoside (3′-deoxy-3′-amino-*N*,*N*-dimethyladenosine), Nutritional Biochemicals Corporation; and N^6-(Δ^2-isopentenyl)adenosine, a gift of Dr. Ross H. Hall. The other nucleotides and nucleosides were obtained from Sigma Chemical Company. Enzymes were obtained either from Sigma or Boehringer-Mannheim (New York).

RESULTS

Previously it was found that 75 μM adenosine inhibited cyclic AMP accumulation due to lipolytic agents in white fat cells (1). The data in Figs. 1 and 2 indicate that only 0.2 μM adenosine was required to give half-maximal inhibition of cyclic AMP accumulation. 5′-AMP and ATP were considerably less active than adenosine as inhibitors of cyclic AMP accumulation (Fig. 1). Inosine 5′-monophosphate and adenosine 2′- or 3′-monophosphate had little effect on cyclic AMP accumulation.[1]

The most potent inhibitor of adenylate cyclase activity in white fat cell ghosts was 2′,5′-dideoxyadenosine, which was 10 times more effective than adenosine (1). Opposite results were seen with respect to inhibition of cyclic AMP accumulation in intact cells, since 2 μM 2′,5′-dideoxyadenosine did not inhibit cyclic AMP accumulation as much as did 0.2 μM adenosine (Fig. 1).

In contrast, phenylisopropyladenosine had little effect on adenylate cyclase activity of fat cell ghosts (1). However, in intact fat cells phenylisopropyladenosine was as effective as adenosine in inhibiting cyclic AMP

[1] J. N. Fain, unpublished observations.

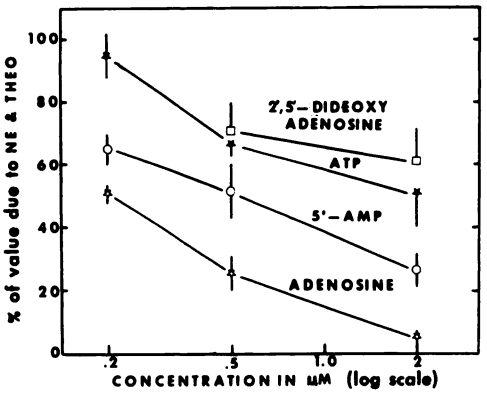

FIG. 1. *Comparison of effects of nucleotides with that of adenosine on cyclic AMP accumulation in the presence of norepinephrine plus theophylline*

Cells were washed twice and incubated (37 mg/tube) for 10–20 min prior to the addition of hormones and drugs. Norepinephrine (NE) (1.5 μM) and theophylline (THEO) (0.1 mM) were added either without or with adenosine or nucleotides, and the reactions were stopped 2 min later. The values in the presence of nucleosides or nucleotides are shown as percentages ± standard errors for four paired experiments. The cyclic AMP accumulation due to norepinephrine and theophylline was approximately 10.6 nmoles/g.

accumulation due to norepinephrine alone (Fig. 2). In the presence of norepinephrine and a small amount of theophylline, phenylisopropyladenosine was about 4 times more active than adenosine (Fig. 2). Phenylisopropyladenosine did not inhibit lipolysis due to norepinephrine, as glycerol release was 9 μmoles/g during 20 min in the absence and 9.5 μmoles/g in the presence of 0.2 μM phenylisopropyladenosine in the experiments shown in Fig. 2.

The greater effectiveness of N^6-(phenylisopropyl)adenosine as contrasted with adenosine was not mimicked by any of the 16 analogues of adenosine which were examined (Table 1). The only adenosine derivative tested which significantly reduced cyclic AMP accumulation was N^6-methyladenosine (Table 1).

The onset of cyclic AMP accumulation due to norepinephrine in the presence of theophylline was quite rapid, with a large increase seen at 20 sec (Fig. 3). Adenosine inhibited cyclic AMP accumulation with equal rapidity. If adenosine was added 1 min after the norepinephrine plus theophylline, the cyclic AMP value failed to increase during the next minute, as it did in the absence of the drug, and 4 min later the value for cyclic AMP accumulation was markedly lower (Fig. 3). In this respect the effect of adenosine was similar to that previously described for prostaglandin E_1 or *beta* adrenergic blocking agents (4, 5).

Insulin can inhibit lipolysis under conditions in which cyclic AMP accumulation is not affected (6, 7). Adenosine, in contrast, markedly inhibits cyclic AMP accumulation

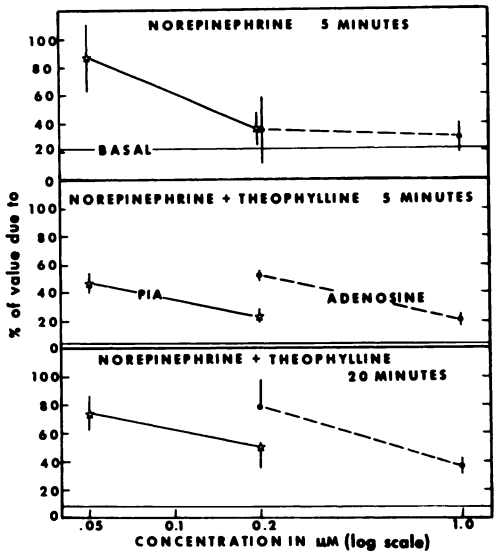

FIG. 2. *Comparison of inhibitory actions of adenosine and phenylisopropyladenosine on cyclic AMP accumulation in fat cells*

Cells were incubated (50 mg/tube) for 10 min prior to the addition of norepinephrine (1.5 μM) in the absence or presence of theophylline (50 μM). Adenosine (●) and N^6-(phenylisopropyladenosine) (PIA, ☆) were added with the lipolytic agents at the indicated concentrations. Cyclic AMP accumulation was measured after 5 or 20 min of incubation. The thin line at the bottom of each graph represents the basal value for cyclic AMP accumulation in the absence of added agents, expressed as a percentage of the value seen with the lipolytic agents. Cyclic AMP accumulation was as follows: basal, 0.1 nmole/g, norepinephrine at 5 min, 0.45; norepinephrine plus theophylline at 5 min, 2.1; and norepinephrine plus theophylline at 20 min, 2.2. The values are for six paired experiments and are the means ± standard errors of the paired differences.

without reducing lipolysis. It was of interest to compare the effects of these two agents. In the studies shown in Fig. 4 insulin inhibited cyclic AMP accumulation to a lesser extent than did adenosine but reduced lipolysis to a much greater degree. In the presence of insulin plus adenosine no further reduction in cyclic AMP accumulation was seen over that found with adenosine alone, while lipolysis was reduced to a greater extent than by insulin alone (Fig. 4).

Both papaverine and dipyridamole are much more effective inhibitors of fat cell phosphodiesterase activity in broken cell preparations than is theophylline (8). These drugs are also able to inhibit the action of adenosine on platelet aggregation (9, 10) and to inhibit adenosine uptake by platelets (9, 10) and red cells (11, 12). In white fat cells neither dipyridamole nor papaverine (50–100 μM) blocked the reduction by adenosine of cyclic AMP accumulation due to norepinephrine (Fig. 5). There was a small enhancement of cyclic AMP accumulation due to 100 μM dipyridamole, but in the same experiments the addition of 100 μM theophylline increased cyclic AMP content to 7 nmoles/g in the presence of norepinephrine. Adenosine at 2 μM did not significantly affect fatty acid release except in the presence of 50 or 100 μM dipyridamole, in which it caused a small but statistically significant reduction. Adenosine was able to reduce cyclic AMP accumulation due to norepinephrine to the basal value when measured at 2 min without

TABLE 1

Effectiveness of various analogues of adenosine as inhibitors of cyclic AMP accumulation

Cells (43 mg/tube) were incubated for 10 min prior to the addition of norepinephrine (1.5 μM) plus theophylline (50 μM). The tubes were then incubated for 20 min with the various nucleosides at a concentration of 1 μM. Cyclic AMP accumulation due to norepinephrine plus theophylline was 1.85 nmoles/g, and basal cyclic AMP accumulation was 9% of this value. The results are shown as percentages ± standard errors of the value for cyclic AMP accumulation (five paired experiments) due to norepinephrine plus theophylline in the absence of added nucleosides.

Nucleoside	Percentage of value seen with norepinephrine + theophylline at 20 min
	%
Adenosine	30 ± 10[a]
N^6-Methyladenosine	50 ± 12[a]
N^6-Dimethyladenosine	84 ± 22
N^6-(Δ^2-Isopentenyl)adenosine	72 ± 30
6-Methoxypurine ribonucleoside	100 ± 22
6-Mercaptopurine ribonucleoside	82 ± 24
6-Mercaptopurine 2′-deoxyribonucleoside	120 ± 40
2′-Deoxyadenosine	110 ± 20
3′-Deoxyadenosine	134 ± 27
9-β-Arabinofuranosyladenine	126 ± 30
2′-O-Methyladenosine	140 ± 20
7-Deazaadenosine (tubercidin)	130 ± 45
Puromycin	74 ± 12
Aminonucleoside of puromycin	120 ± 50
Guanosine	74 ± 11
2′-Deoxyguanosine	90 ± 30
2′-Deoxyinosine	102 ± 27

[a] Significant effect of added nucleoside ($p < 0.05$).

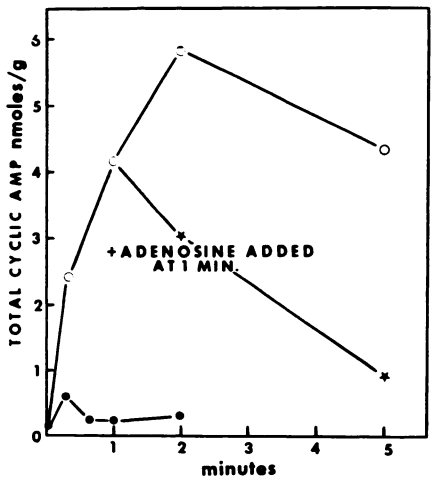

FIG. 3. *Onset of adenosine inhibition in the presence of norepinephrine and theophylline*

Fat cells (40 mg/tube) were incubated for 10–20 min prior to the addition of hormones and drugs. Norepinephrine (1.5 μM) and theophylline (0.1 mM) were added either without or with adenosine (2 μM), and cyclic AMP accumulation was measured for periods from 20 sec to 5 min. The values shown are the means of four experiments: ★, adenosine was added 60 sec after the lipolytic agents; ●, adenosine was added with norepinephrine and theophylline; ○, norepinephrine and theophylline.

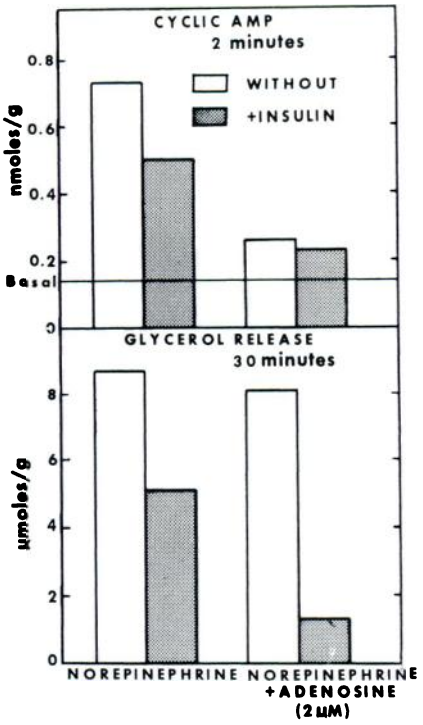

FIG. 4. *Dissociation between effects of insulin and adenosine on lipolysis as contrasted with cyclic AMP accumulation*

Cells (30 mg/tube) were incubated for 10 min in the absence or presence of insulin (120 microunits/ml). Norepinephrine (1.5 μM) was then added either without or with adenosine (2 μM). Cyclic AMP accumulation was measured after 2 min, and glycerol release after 30 min. The values shown are the means of six paired experiments. The basal value for cyclic AMP was 0.14 nmole/g (shown by the thin line across the figure marked basal), and for glycerol release it was undetectable. The mean ± standard error of the paired difference for the effect of insulin on cyclic AMP was −0.23 ± 0.09, and for glycerol release, −3.6 ± 1.1. The decrease in cyclic AMP accumulation due to adenosine was −0.48 ± 0.08, and in glycerol release, −0.6 ± 0.2. The decrease in glycerol release due to adenosine in the presence of insulin was −3.0 ± 0.7 over that seen with insulin alone. The increases in cyclic AMP and glycerol due to norepinephrine were 0.59 ± 0.12 and 8.7 ± 1.3, respectively. In the presence of adenosine the increases in cyclic AMP and glycerol due to norepinephrine were 0.12 ± 0.09 and 8.1 ± 1.1. In the presence of adenosine and insulin the increases in cyclic AMP and glycerol due to norepinephrine were 0.09 ± 0.04 and 1.3 ± 0.3.

reducing lipolysis over a 30-min period (Fig. 5). Fatty acid release rather than glycerol release was used as the index of lipolysis in the presence of dipyridamole, because of interference of the drug with glycerol analysis.

The possibility that adenosine was specific as an inhibitor of catecholamine-induced in-

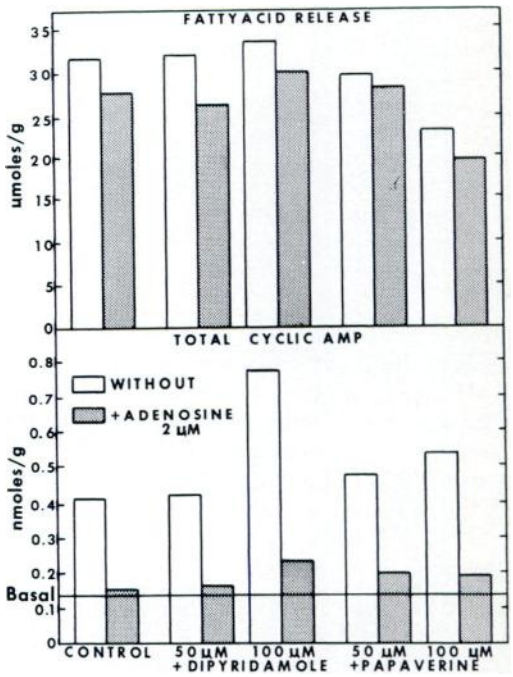

FIG. 5. *Failure of papaverine or dipyridamole to block action of adenosine*

Fat cells (35 mg/tube) were incubated for 10 min in the absence or presence of dipyridamole (50 or 100 μM) or papaverine (50 or 100 μM). Norepinephrine (1.5 μM) was added either without or with adenosine (2 μM). Cyclic AMP accumulation was measured after 120 sec, and fatty acid release after 30 min. The values shown are the means of five paired experiments. The basal value for cyclic AMP accumulation in the absence of norepinephrine was 0.14 nmole/g. The decrease caused by adenosine in norepinephrine-induced fatty acid release, as the mean ± standard error of the paired differences, was 4.2 ± 1.9 in the absence and 5.9 ± 2.0 and 3.8 ± 0.9 in the presence of 50 and 100 μM dipyridamole, respectively. The decrease in fatty acid release due to papaverine was 8.4 ± 2.2 in the absence and 8.2 ± 2.5 in the presence of adenosine. The increase in fatty acid release due to norepinephrine was 32.1 ± 2.5, and in cyclic AMP, 0.4 ± 0.05.

TABLE 2

Effect on adenosine action of omission of Ca^{2+} from incubation medium

Cells (15 mg/tube) were incubated for 10 min in regular buffer or in buffer without added Ca^{2+} in the presence of 0.25 mM EGTA. Then norepinephrine, theophylline, or adenosine was added, and the cells were incubated for 5 min. The values are the means of five experiments, with the increment (or decrement) due to adenosine (1 μM) shown as the mean ± standard error of the paired differences.

Conditions	Regular buffer		Ca^{2+}-free +0.25 mM EGTA	
	Basal	Δ due to adenosine	Basal	Δ due to adenosine
	nmoles cyclic AMP/g		*nmoles cyclic AMP/g*	
Zero time	0.17		0.20	
5-min control	0.35		0.28	
+Norepinephrine, 0.15 μM	0.65		1.65	
+Norepinephrine, 1.5 μM	1.32	−0.88 ± 0.25	1.44	−0.49 ± 0.16
+Norepinephrine, 0.15 μM, + theophylline, 50 μM	8.95	−8.37 ± 2.97	13.6	−12.2 ± 4.1
+Norepinephrine, 1.5 μM, + theophylline, 50 μM	5.87	−4.61 ± 1.30	11.9	−10.9 ± 3.0

creases in cyclic AMP was excluded by the finding that adenosine also inhibited cyclic AMP accumulation induced by glucagon. The addition of 1 μg/ml of glucagon in the presence of 0.1 mM theophylline increased cyclic AMP accumulation from a basal value of 0.1 nmole/g to 1.5 nmoles/g after 2 min and to 2.2 nmoles/g after 5 min. In the presence of 2 μM adenosine cyclic AMP accumulation at either time interval was less than 0.2 nmole/g.

The ability of adenosine to inhibit methylxanthine-induced stimulation of heart muscle has been attributed to effects on Ca^{2+} flux (13, 14). However, the ability of 1 μM adenosine to inhibit cyclic AMP accumulation due to norepinephrine in both the presence and absence of theophylline was unimpaired in the absence of calcium. Fat cells were isolated in buffer without added Ca^{2+} and then incubated in the same buffer in the presence the Ca^{2+} chelator EGTA[2] (0.25 mM). The results indicate that the effects of both norepinephrine and theophylline on cyclic AMP accumulation, as well as that of adenosine, do not require the presence of Ca^{2+} in the medium or any pool of Ca^{2+} which is accessible to EGTA (Table 2). Essentially the same maximal rate of fatty acid release was produced in both media by 0.15 μM norepinephrine. There was no significant decrease by adenosine under any conditions.

If adenosine is released during incubation of fat cells it should be possible to inhibit its accumulation by accelerating its removal by deamination. Addition of 0.5 μg of purified adenosine deaminase from calf intestine (200 units/mg) increased basal cyclic AMP accumulation and lipolysis in fat cells (Table 3). The lowest concentration of norepinephrine tested in the studies shown in Table 3 produced maximal activation of lipolysis without affecting cyclic AMP accumulation in the absence of adenosine deaminase. However, in the presence of added deaminase the addition of 0.075 μM norepinephrine markedly increased cyclic AMP accumulation (Table 3). Adenosine deaminase also potentiated the increase in cyclic AMP due to 1.5 μM norepinephrine in the presence of theophylline. Tests of the deaminase preparation for activity prior to use at 37° revealed that 0.5 μg/ml would deaminate 0.33 μmole of adenosine per minute at an adenosine concentration of 100 μM. The addition of 5 μg/ml of adenosine deaminase actually had a smaller effect on cyclic AMP accumulation and lipolysis than did 0.5 μg/ml.[1]

The increase in cyclic AMP due to

[2] The abbreviation used is: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

TABLE 3

Effect of adenosine deaminase on lipolysis and cyclic AMP accumulation in isolated white fat cells

Cells (50 mg/tube) were incubated for 10 min either without or with added crystalline adenosine deaminase (0.5 μg/ml, 200 units/mg, from calf intestinal mucosa, Sigma lot 30C-2643-85). After 10 min norepinephrine and/or theophylline were added, and the incubation was continued for 2 or 20 min. The values are the means of five experiments, and the effect of 5 μg/ml of adenosine deaminase is shown as the mean ± standard error of the paired differences.

Additions	Cyclic AMP at 2 min		Cyclic AMP at 20 min		Glycerol released at 20 min	
	Control	Δ due to adenosine deaminase	Control	Δ due to adenosine deaminase	Control	Δ due to adenosine deaminase
	nmoles/g		*nmoles/g*		*μmoles/g*	
None	0.33	+0.27 ± 0.11	0.15	+0.25 ± 0.06	0.4	+1.5 ± 0.6
Norepinephrine, 0.075 μM	0.10	+1.10 ± 0.35	0.24	+1.10 ± 0.40	6.2	+4.8 ± 1.7
Norepinephrine, 0.15 μM	0.26	+1.80 ± 0.45	0.22	+1.62 ± 0.42	4.9	+1.3 ± 1.1
Norepinephrine, 1.5 μM	0.22	+1.40 ± 0.50	0.33	+1.79 ± 0.60	8.3	−0.9 ± 1.2
Norepinephrine, 1.5 μM, + theophylline, 100 μM	0.92	+3.48 ± 0.65	0.81	+1.40 ± 0.52	6.2	+2.7 ± 2.0

norepinephrine seen at 2 min in the presence of adenosine deaminase was maintained over the 20-min incubation period (Table 3). The effect of adenosine deaminase was also equally apparent at both time intervals. These observations provide indirect evidence for the release of adenosine by incubated fat cells if one assumes that the effects of the added enzyme are manifested solely by adenosine deamination.

DISCUSSION

Adenosine was found to be a potent inhibitor (50% blockade with 0.2 μM or less) of cyclic AMP accumulation due to norepinephrine in both the absence and presence of methylxanthines under conditions in which it did not affect lipolysis. However, the present results do not contradict the hypothesis that cyclic AMP is the mediator for catecholamine-induced lipolysis, since the increase in cyclic AMP needed to activate lipolysis may be so small as to be unmeasurable.

The present results are in agreement with those of Schwabe *et al.* (15). Adenosine was a potent inhibitor of cyclic AMP accumulation due to norepinephrine in white fat cells, with 5′-AMP being less effective, while ATP, ADP, inosine, and hypoxanthine were inactive (15).

Schwabe and Ebert (16) had earlier found that if 4 mg/ml of white fat cells were incubated with 1 μM isoproterenol a marked increase in cyclic AMP accumulation was seen, which was not further enhanced by 1 mM theophylline. In contrast, when 20 mg/ml of fat cells were incubated with isoproterenol alone, there was little increase in cyclic AMP unless 1 mM theophylline was present (16). Schwabe and Ebert (16) postulated that an inhibitor of cyclic AMP accumulation is released when large amounts of fat cells (20 mg/ml or more) are incubated. Subsequently they were able to show that an inhibitor of cyclic AMP accumulation was being released, which appeared to be adenosine (15). Schwabe *et al.* (15) based the same conclusion on the finding that the inhibitor was a chloroform-insoluble, heat-stable substance of low molecular-weight, present in the nucleoside fraction obtained by gel filtration, which traveled in the same position as adenosine during thin-layer chromatography. Treatment of the inhibitor with adenosine deaminase destroyed its activity (15). If 70 mg/ml of fat cells were incubated for 10 min, about 7 nmoles of adenosine were released per gram of fat cells. This was increased to only 9 nmoles/g by the addition of 10 μM norepinephrine (15).

Many drugs are much more potent inhibitors of cyclic AMP phosphodiesterase

than methylxanthines but do not mimic their effects on lipolysis (6, 17). Similar results have been shown in the present report, since dipyridamole and papaverine, which are potent inhibitors of the cyclic AMP phosphodiesterase activity of fat cell homogenates (6), had little effect on cyclic AMP accumulation in intact cells. Dipyridamole and papaverine (100 μM) were effective inhibitors of basal and insulin-stimulated glucose oxidation in fat cells,[1] which indicates that they are able to affect the metabolism of intact cells. Papaverine (100 μM) was a potent stimulator of aerobic glycolysis in fat cells[1] and slightly inhibited fatty acid release (Fig. 5), which is compatible with the suggestion that its primary affect on fat cells is to inhibit mitochondrial respiration (17).

Another reason for examining the effects of dipyridamole and papaverine is that these drugs prevent adenosine uptake and incorporation into nucleotides by red cells (9, 10) and platelets (7, 8, 18). However, prevention of adenosine uptake into platelets by papaverine (7) or dipyridamole (18) actually enhanced the inhibition of platelet aggregation due to adenosine. Mills and Smith (19) found that adenosine, like prostaglandin E_1, increased cyclic AMP accumulation in platelets. Why adenosine should be a potent activator of cyclic AMP accumulation in platelets (20) and brain slices (21) while having just the opposite effect on white fat cell cyclic AMP is unclear.

The relationship of adenosine action to that of prostaglandin E_1 is not known. Both compounds inhibit the increase in cyclic AMP accumulation due to catecholamines (17) in fat cells but increase cyclic AMP accumulation in platelets (20) and brain slices (21).

The remarkable potency of N^6-(phenylisopropyl)adenosine as an inhibitor of cyclic AMP accumulation may be due to its greater intrinsic activity than adenosine and its resistance to inactivation by adenosine deaminase because of the absence of a primary amino group at position N^6. Phenylisopropyladenosine was not deaminated by adenosine deaminase from calf intestine, nor did it inhibit deamination of adenosine by the enzyme.[1] Resistance to inactivation by deaminase probably explains why the administration to humans of phenylisopropyladenosine, but not adenosine, results in inhibition of fatty acid mobilization (22).

Westermann and associates (23, 24) reported that phenylisopropyladenosine was much more effective in blocking lipolysis due to catecholamines than that due to theophylline. However, we previously found 5 μM phenylisopropyladenosine to be an extremely effective inhibitor of lipolysis due to 0.1 mM theophylline, but less effective in reducing the increase in lipolysis due to 1.5 μM norepinephrine (1). Phenylisopropyladenosine at 0.2 μM did not block lipolysis due to 1.5 μM norepinephrine in the present studies.

Perhaps phenylisopropyladenosine, like adenosine, blocks the physiologically important increase in cyclic AMP due to low concentrations of catecholamine, and this results in inhibition of lipolysis. When larger amounts of catecholamine are added, the inhibition of cyclic AMP accumulation is insufficient to reduce lipolysis but is enough to prevent the accumulation of measurable amounts of cyclic AMP over the basal value. It is also possible that factors other than cyclic AMP are involved in maintenance of the lipolytic action of catecholamines.

One unexpected finding was the lack of correlation between effects of nucleosides on adenylate cyclase activity of white fat cells (1) and cyclic AMP accumulation in intact fat cells in the presence of norepinephrine. Why dideoxyadenosine, which was the most potent inhibitor of adenylate cyclase, was relatively ineffective in intact fat cells remains to be elucidated. Phenylisopropyladenosine was a much more effective inhibitor of cyclic AMP accumulation than of adenylate cyclase. However, the cyclase assay is performed in the presence of fairly high concentrations of ATP and under other conditions not present in the intact fat cell. The results do indicate that one cannot assume that the effects of nucleosides on adenylate cyclase activity of broken cell preparations reflect their effects on cyclic AMP accumulation in intact cells.

The finding that Ca^{2+} has little effect on lipolysis or the action of adenosine is hardly

surprising. Khoo *et al.* (25) have similarly found that omission of Ca^{2+} from the buffer used for isolation and incubation of fat cells, even in the presence of 1 mM EGTA, did not affect the increase in cyclic AMP, phosphorylase activity, or lipase activation by protein kinase in fat cell extracts seen after incubation of fat cells for 5 min with 0.3 μM epinephrine. There does not seem to be the same relationship between Ca^{2+} and lipolysis as there is between adenosine, methylxanthines, and Ca^{2+} and cardiac contractility (13, 14). Adenosine action clearly is not mediated by any process in fat cells which requires extracellular Ca^{2+} or a Ca^{2+} pool which is accessible to EGTA.

The action of insulin on lipolysis and cyclic AMP accumulation was quite different from that of adenosine. The greater inhibition of lipolysis by insulin, with a smaller effect on cyclic AMP accumulation, suggests that the antilipolytic action of insulin is not secondary to changes in cyclic AMP accumulation. Butcher *et al.* (26) originally reported that insulin did not reduce the small increase in cyclic AMP accumulation due to catecholamines alone in isolated fat pads but did reduce that seen in the presence of catecholamines and methylxanthines. There is increasing evidence that although insulin can affect cyclic AMP content under some circumstances there is no correlation between insulin action on cyclic AMP content and lipolysis (6, 7, 25).

These studies indicate that adenosine is released during incubation of isolated fat cells and acts to inhibit adenylate cyclase activity. There does not yet appear to be any evidence that adenosine release is specifically regulated by catecholamines or other hormones, but it is possible that some agents may influence fat cell metabolism by affecting adenosine formation, degradation, or action. Whether adenosine is physiologically important as a regulator of lipolysis remains to be established. The abilities of added adenosine at low concentrations to inhibit cyclic AMP accumulation and of adenosine deaminase to increase cyclic AMP accumulation provide further support for the hypothesis that adenosine or related substances may be important in the regulation of fat cell metabolism.

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