STIMULATION OF RAT FAT CELL PHOSPHODIESTERASE BY ADENOSINE

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1. Introduction

There is considerable interest in the role of adenosine on the regulation of fat cell metabolism. It has been shown to antagonize cyclic AMP accumulation and inhibit lipolysis in a manner similar to that of prostaglandin E and nicotinic acid [1–9]. The addition of adenosine deaminase to fat cells potentiates cyclic AMP accumulation and increases lipolysis caused by lipolytic hormones and abolishes the antilipolytic activity of insulin [6,7]. Its inhibitory effects on hormone-stimulated cyclic AMP production is, however, greater than its effects on the increase in lipolysis [8,10]. Since the maximal rates of lipolysis require a relatively small increase in cyclic AMP concentration [10,11], the important role of adenosine may be to prevent a large increase in the concentration of cyclic AMP in fat cells rather than the inhibition of lipolysis per se [12].

Cyclic AMP is produced from ATP in a reaction catalyzed by adenylate cyclase and, degraded by the enzyme cyclic AMP phosphodiesterase. Changes in the level of cyclic AMP could result from changes in the rate of one or both the reactions. In fat cells, the concentration of cyclic AMP rises to a peak within a few minutes of hormone stimulation and declines rapidly thereafter towards basal levels [13,14]. The rapid decline could be due to an inhibition of adenylate cyclase, as well as a stimulation of cyclic AMP phosphodiesterase. Adenosine has been shown to inhibit adenylate cyclase activity of fat cell ghosts [1,15] and of purified membranes of rat fat cells [16]. The evidence for the involvement of adenosine on phosphodiesterase has, on the other hand, been obtained indirectly by the study of the effects of papavarine and dipyridamole, inhibitors of adenosine transport, and caffeine and theophylline, which block adenosine effects. Here, we show that adenosine activates cyclic AMP phosphodiesterase in rat fat cells. We propose that the effect of adenosine in reducing the intracellular concentration of cyclic AMP could be due to the concerted action of adenosine in simultaneously inhibiting adenylate cyclase and activating cyclic AMP phosphodiesterase.

2. Materials and methods

Most chemicals including bovine serum albumin (fraction V), adenosine, collagenase (type II), cyclic AMP, 5′-nucleotidase from *Crotalus adamanteus* venom (77 units/mg protein) and Hepes were obtained from Sigma. Labeled cyclic [^{3}H]AMP (37.7 Ci/mmol) was obtained from the Radiochemical Centre, Amersham.

Isolated fat cells were prepared by the collagenase method in [17] from the epididymal adipose tissues of fed male Sprague-Dawley rats (160–200 g). Krebs-Henseleit-Hepes buffer (pH 7.4) containing 2.5 mM calcium and 4% bovine serum albumin was used. Tissue from several animals were pooled to reduce the variability seen among individual animals.

Unless otherwise noted, the fat cells were distributed into separate polyethylene vials, gassed with oxygen, and the vials closed tightly with rubber stoppers. Cells were not preincubated before the start of experiments to minimise the production of adenosine by the cells. Experiments were usually done for 10 min at 37°C, after which the fat cells were immediately homogenised by means of Dounce tissue grinders (Type B pestle, 8 strokes). The homogenates were then centrifuged at 3000 rev./min (800 × *g*) for 3 min and the infranatants assayed for phosphodiesterase activity.

Cyclic AMP phosphodiesterase assays were performed with a final volume of 0.8 ml containing

80 mM Tris–HCl (pH 8.0), 3 mM magnesium acetate, 0.2 mM $CaCl_2$, 0.13 μCi cyclic [^{3}H]AMP and 0.1 μM unlabelled cyclic AMP. The assay was done at 30°C for 10 min and the reaction terminated by freezing the tubes in a dry-ice–ethanol bath. In all the experiments, the amount of enzyme added caused hydrolysis of <15% of the substrate. The frozen tubes were then placed in a boiling water bath for 90 s, cooled and to each and every tube was added 0.2 units of 5′-nucleotidase, adenosine, to a final concentration of 20 μM, and 10 μM 5′-AMP, and the tubes incubated at 30°C for a further 20 min. EDTA (0.1 ml, 50 mM) was added to stop the reaction. Tritiated adenosine was separated from the unreacted cyclic AMP by the method in [18] as modified [19]. By this method, the reaction mixtures were passed over a column (0.7 × 2.5 cm) of quarternary aminoethyl (QAE)–Sephadex (A-25) in the formate form. The tubes were rinsed with 1 ml 20 mM ammonium formate (pH 7.4) which was also passed over the column, followed by 3 ml of the same ammonium formate solution. The effluent (5 ml) was collected in glass scintillation vials and counted in 15 ml of a triton-based scintillation mixture.

Dry weights of isolated fat cells were determined by pipetting aliquots (0.5 ml) of the fat cell suspension onto tared (25 mm Millipore filter paper (SMWP 02500) pre-moistened with water. The medium was removed by vacuum filtration and the filter paper dried over silica gel under reduced pressure and reweighed. The average of 3 determinations were used as the cell weight.

Results from representative experiments are shown. Data are reported as the mean of duplicate or triplicate incubations, which in all cases were within 10% of each other. All observations were confirmed by repetition on at least 3 other occasions using different batches of cells. The validity of expressing the results in this manner, because of the variability in the degree of response from different batches of cells, has been discussed [20–22].

3. Results and discussion

3.1. *Time course effect of adenosine on cyclic AMP phosphodiesterase*

The failure to observe any effects of a compound on the activity of an enzyme could have been due to the transient nature of its effects. A study of the time course effect of adenosine on the cyclic AMP phosphodiesterase was therefore made. Adenosine was used at 20 μM in the experiments because it was shown in [8] that 10 μM adenosine had a maximum inhibitory effect on the accumulation of cyclic AMP in the presence of norepinephrine. For good measure, twice this concentration was used.

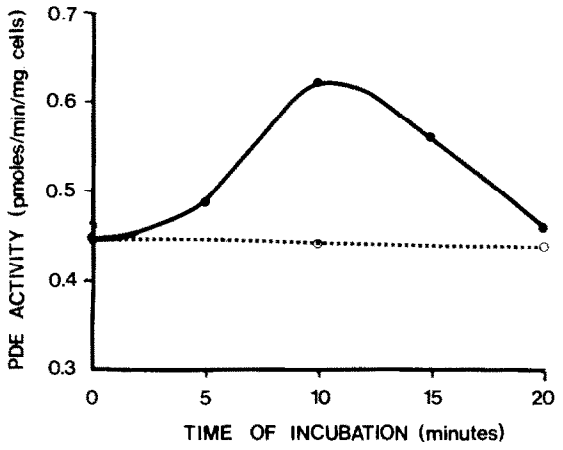

Fig.1. Time course effect of adenosine on intact cell phosphodiesterase. Fat cells (8 mg/ml) were added to 20 μM adenosine and incubated at 37°C for the length of time indicated.

Fig.1 shows that 20 μM adenosine stimulated the activity of the low K_m cyclic AMP phosphodiesterase of rat fat cells. Maximum stimulation was attained within 10 min after the addition of adenosine, followed by a rapid decline towards basal activity. The pattern of the time course effect of adenosine stimulation of phosphodiesterase, was consistent with the observation that in fat cells, cyclic AMP accumulation reaches a peak within a short period of hormone stimulation, after which there is a rapid decline presumably due to the concerted action of cyclic AMP phosphodiesterase and the inhibition of adenylate cyclase activity.

3.2. *Effect of adenosine concentration on the stimulation of cyclic AMP phosphodiesterase*

Adenosine nucleosides inhibited the low K_m phosphodiesterase found in the supernatant fraction obtained after centrifugation of rat fat cell homogenates [1]. The inhibitory rather than stimulatory effect observed could have been due to the high concentration of adenosine used, or, the observations in [1], made on the cyclic AMP phosphodiesterase in a cell-free system, differed from our experiments with

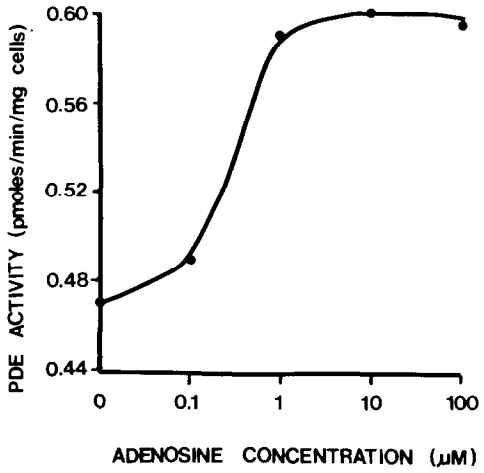

Fig.2. Effect of adenosine concentration on intact cell phosphodiesterase. Fat cells (4 mg/ml) were added to various concentrations of adenosine and incubated at 37°C for 10 min.

intact cells. Experiments were done with varying amounts of adenosine on phosphodiesterase from intact cells, as well as on phosphodiesterase from broken cells (fig.2,3).

Adenosine as low as 0.1 μM had a stimulatory effect on phosphodiesterase in intact cells (fig.2). This effect increased with increasing adenosine concentrations reaching a maximum at 10 μM. The concentration of adenosine at which maximum stimulation of phosphodiesterase activity was achieved, however, varied from one cell preparation to another. At dilute cell concentrations (<10 mg/ml), maximum stimulatory effect was obtained with 10 μM adenosine (fig.2). The stimulatory effect of adenosine, however, appeared to diminish slightly at 100 μM but was never

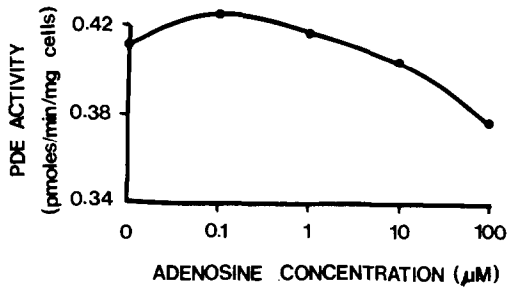

Fig.3. Effect of adenosine concentration on broken cell phosphodiesterase. Fat cells (6 mg/ml) were homogenised and the phosphodiesterase activity determined in the presence of various concentrations of adenosine.

Table 1
Cell concentration and the effect of adenosine on phosphodiesterase activity

[Fat cell] (mg/ml)	PDE act. (pmol . min^{-1} . mg cells^{-1})		Activation (%)
	Control	20 μM Adenosine	
7	0.146 ± 0.003	0.237 ± 0.006	62%
42	0.140 ± 0.007	0.192 ± 0.001	38%

Values represent the mean ± SEM

found to be inhibitory. The effect of adenosine on phosphodiesterase from homogenates of fat cells not pre-exposed to adenosine were different (fig.3). Low concentrations of adenosine were found to have little or no effect on the phosphodiesterase activity. The higher concentrations had an inhibitory effect similar to that observed in [1] with 70 μM adenosine, on the phosphodiesterase activity of the 48 000 × *g* supernatant, obtained by centrifugation of fat cell homogenates. Thus it would appear that the stimulatory effect of adenosine on fat cell phosphodiesterase can be demonstrated only with intact cells as is the case with insulin.

3.3. *Cell concentration and the effects of adenosine*

Adenosine is continously released by fat cells into the incubation medium in amounts which inhibit cyclic AMP accumulation and lipolysis due to norepinephrine [3]. This inhibitory effect was diminished when dilute cell suspensions containing 20 000 fat cells/ml were used [23]. Adenosine deaminase enhanced the basal lipolytic activity of fat cells, and the effect was more pronounced at low cell concentrations than at concentrated cell suspensions. Thus the stimulatory effect of adenosine on the cyclic AMP phosphodiesterase should be greater at low cell concentrations than with high cell concentrations. This was in fact found to be the case (table 1). Controls were done for each cell concentration used since the amount of adenosine released is known to increase with increasing cell concentrations [23]. A lowering of basal phosphodiesterase activity could not be demonstrated with adenosine deaminase.

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