EFFECT OF HORMONES ON CYCLIC AMP LEVELS IN CULTURED HUMAN CELLS

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SUMMARY

Cultured cells derived from human adipose tissue grew more slowly and had significantly higher basal levels of cyclic AMP than cultured fibroblasts. Cyclic AMP levels in cultured adipose tissue cells were unaffected by epinephrine and were elevated 15-fold by prostaglandin E_1 while fibroblast cyclic AMP levels were elevated 27-fold by epinephrine and 95-fold by prostaglandin E_1. These results support the postulate that the cultured adipose tissue cell is a distinct cell type which may represent an adipocyte or preadipocyte in culture.

INTRODUCTION

Cultured cells derived from human adipose tissue (CAT cells)[1] retained intracellular lipid and had a significantly slower growth rate than cultured fibroblasts (1). Although CAT cells incorporated more labeled glucose into lipid than fibroblasts, to date we have not been able to unequivocally identify these cells as either cultured adipocytes or preadipocytes. Adipose cells have been shown to respond to hormones such as epinephrine and insulin with characteristic changes in their levels of cyclic AMP (2). We have, therefore, investigated cyclic AMP levels and their response to hormones in both CAT cells and fibroblasts in order to more fully characterize the metabolic differences between the two cell lines.

MATERIALS AND METHODS

Cell Culture: Subcutaneous adipose tissue was obtained from subjects undergoing abdominal surgery, and CAT cell cultures were established as previously described (1). Fibroblasts and CAT cells were cultured in RPMI-1640 medium supplemented with 20% fetal calf serum, glutamine (0.3 mg/ml), penicillin (100 U/ml) and streptomycin (100 µg/ml). Cells were fed twice weekly and subcultured with 0.5% trypsin in Puck's saline A. For the determination of cellular growth rate, CAT cells and fibroblasts were subcultured, and rep-

[1]Abbreviations used: CAT cells, cultured cells derived from adipose tissue; PGE_1, prostaglandin E_1.

licate flasks containing 1.8 x 10^5 cells were harvested by trypsinization on subsequent days following the initial subculture, and the total number of cells per flask was determined by counting in a Coulter cell counter.

Cyclic AMP Determination: All determinations of cyclic AMP levels were made on cells between the second and fifteenth passage following the initial subcultivation. Confluent flasks were subcultured, and replicate flasks with appropriate cell numbers were established. Fibroblasts and CAT cells were diluted so as to have equivalent numbers of cells in the experimental flasks to be compared. On the second to third day after trypsinization the growth medium was removed, and the cultures were incubated for one hour at 37°C with serum-free medium in an atmosphere of 95% O_2-5% CO_2. Hormones were added as indicated, and the cells were incubated at 37°C for the specified time. At the end of the incubation period the medium was removed, the cell monolayer was washed once with saline, and cyclic AMP was extracted with cold 10% trichloroacetic acid. The cells were scraped from the flasks, and the medium plus cells were centrifuged. The cell precipitate was analyzed for DNA content by the diphenylamine method (3). The supernatant was extracted three times with water-saturated ether, heated at 70°C for 5 minutes and lyophilized. The lyophilized material was taken up in 0.05 M sodium acetate buffer (pH 6.2), appropriately diluted and analyzed for cyclic AMP content by the radioimmunoassay method of Steiner et al (4) using biochemicals obtained from Schwarz/Mann.

Insulin (crystalline porcine) was supplied by Eli Lilly Company and L-epinephrine was obtained from Parke-Davis. PGE_1 was supplied by Dr. John E. Pike of the Upjohn Company.

RESULTS AND DISCUSSION

Basal intracellular levels of cyclic AMP were determined in cultures of actively growing fibroblasts (5 cell lines) and CAT cells (4 cell lines). The CAT cells had significantly higher basal levels of cyclic AMP than fibroblasts

TABLE I. Effect of hormones on cyclic AMP levels in CAT cells and fibroblasts.

	Intracellular cyclic AMP levels (pmoles/μg DNA)		
Additions	CAT Cells	Fibroblasts	p
None	1.02 ± 0.241 (14)	0.39 ± 0.071 (17)	$p < 0.02$
Epinephrine (6.8μM)	1.24 ± 0.220 (13)	10.61 ± 1.946 (16)	$p < 0.01$
Epinephrine (6.8μM) and insulin (1mU/ml)	0.89 ± 0.093 (12)	9.71 ± 2.059 (14)	$p < 0.01$
PGE_1 (5μM)	15.06 ± 3.716 (6)	37.26 ± 10.730 (6)	$p < 0.10$

Cells were incubated for 5 min. at 37° in the presence or absence of hormones. The incubation was terminated, and intracellular cyclic AMP levels were determined as described in Materials and Methods. Results are expressed as the mean ± S. E. M. for (n) experiments. The p column represents the significance of the difference between CAT cells and fibroblasts for each experimental condition.

(Table I). The growth rate of the CAT cells was much slower than that of fibroblasts. Comparative growth curves of a fibroblast line and a CAT cell line derived from the same individual are shown in Fig. 1. Otten et al (5), in a study of 13 different cell lines, found that intracellular levels of cyclic AMP were inversely proportional to the cell growth rate, and our findings support this correlation.

Epinephrine caused a significant rise in cyclic AMP levels in fibroblasts (Table I). There was a 27-fold increase in cyclic AMP in fibroblasts after a five minute incubation with epinephrine. No significant stimulation of cyclic AMP levels by epinephrine was observed in the CAT cells. Insulin (1 mU/ml) had no effect on epinephrine-stimulated cyclic AMP levels in fibroblasts (Table I). There was also no significant difference in the levels of cyclic AMP in CAT cells incubated with epinephrine and insulin compared to those with epinephrine alone. Cyclic AMP levels in fibroblasts and CAT cells derived from the same subject were compared after varying times of incubation with epinephrine (Fig. 2). Maximal stimulation of cyclic AMP levels in fibroblasts occurred at five minutes. By ten minutes the levels of cyclic AMP had

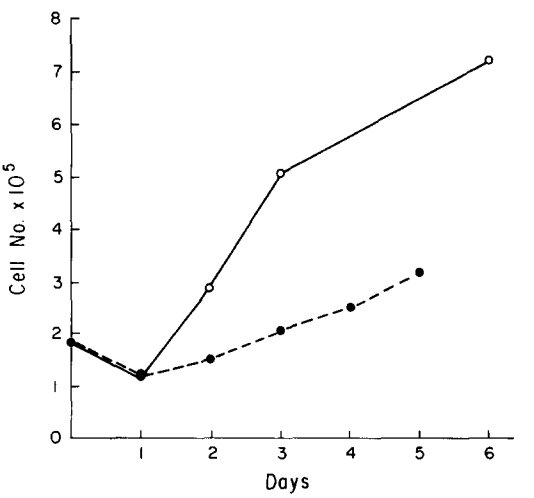

Fig. 1. Comparative growth rates of fibroblasts (O-O) and CAT cells (●--●) derived from the same individual. Total cell numbers were determined on subsequent days following trypsinization and plating as described in Materials and Methods.

fallen to about twice basal levels and remained only slightly elevated from ten to thirty minutes. Cyclic AMP levels in CAT cells incubated with epinephrine remained essentially unchanged up to thirty minutes. The response of fibroblasts to epinephrine has been reported to be markedly influenced by cell density. Fibroblasts at lower cell densities were found to respond to epinephrine with larger increases in cyclic AMP levels than cells at higher densities or at confluence (6). The present experiments, demonstrating large increases in cyclic AMP in response to epinephrine, used fibroblasts at relatively low cell densities. We also have observed that confluent fibroblasts respond to epinephrine with only small increases in cyclic AMP. The differences in epinephrine-stimulated levels of cyclic AMP found between fibroblasts and CAT cells were apparently not related to cell density since comparisons were made between the two cell types using flasks containing equal numbers of cells, and confluent cultures of CAT cells also did not respond to epinephrine (unpublished observations).

PGE_1 raised cyclic AMP levels 95-fold in fibroblasts and 15-fold in CAT cells in a five minute incubation period (Table I). The time course of the response of cyclic AMP levels to PGE_1 by fibroblasts and CAT cells derived from the same subject is shown in Fig. 3. The response of fibroblasts to PGE_1

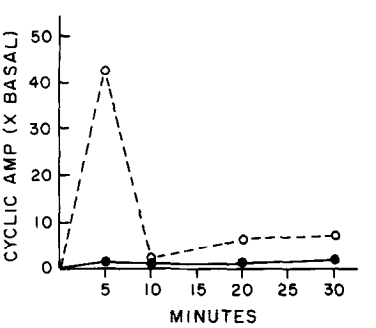

Fig. 2. Effect of epinephrine (6.8 μM) on cyclic AMP levels in fibroblasts (O--O) and CAT cells (●-●) after varying incubation periods (5-30 min.). Incubations were carried out as described in Materials and Methods, and results are expressed as levels of cyclic AMP times basal levels. The fibroblast and CAT cell lines were derived from the same individual.

was maximal at five minutes. From ten to thirty minutes cyclic AMP levels declined somewhat but remained significantly above the levels in untreated control cells. Cyclic AMP levels in CAT cells were maximally stimulated by PGE_1 at ten minutes and remained significantly elevated from ten to thirty minutes.

Cyclic AMP levels in human fibroblasts were significantly elevated by epinephrine and PGE_1. The magnitude and time course of the hormonal effects on cyclic AMP levels found in these studies were comparable to those reported by others (6-8). CAT cells derived from human adipose tissue had significantly higher basal levels of cyclic AMP than fibroblasts. The CAT cells did not respond to epinephrine in terms of increased cyclic AMP levels. The cyclic AMP levels of isolated human fat cells have been reported to be only slightly elevated (1½-fold) by epinephrine (9). Insulin had no significant effect on the epinephrine-stimulated cyclic AMP levels in fibroblasts or CAT cells during a five minute incubation. Insulin has been shown to lower cyclic AMP levels elevated by the addition of epinephrine and methyl xanthines in rat fat cells (2), but it does not significantly alter basal or stimulated cyclic AMP levels in

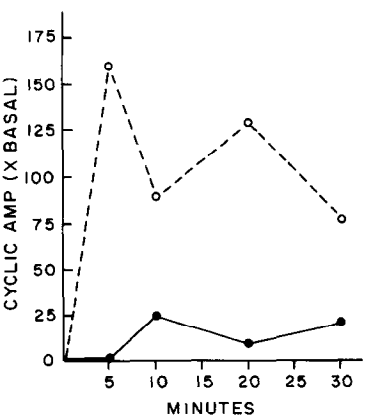

Fig. 3. Effect of PGE_1 (5μM) on cyclic AMP levels in fibroblasts (0--0) and CAT cells (●-●) after varying incubation periods (5-30 min.). Incubations were carried out as described in Materials and Methods, and results are expressed as levels of cyclic AMP times basal levels. The fibroblast and CAT cell lines were derived from the same individual.

human fibroblasts (8). Different experimental conditions (e.g., longer incubation times, different insulin concentrations) may be necessary in order to demonstrate an effect of insulin on cyclic AMP levels in CAT cells.

PGE_1 raised cyclic AMP levels in CAT cells but to a much lesser extent than in fibroblasts. PGE_1 has been shown to have an antilipolytic action on isolated human fat cells (10). In isolated rat fat cells PGE_1 did not elevate cyclic AMP levels and antagonized the stimulatory effect of epinephrine on cyclic AMP (11). To date there have been no reports in the literature on the effects of PGE_1 on cyclic AMP levels in isolated human fat cells. Preliminary studies in our laboratory have shown that, at least in some individuals, PGE_1 slightly elevated cyclic AMP levels in human fat cells, and further experiments are presently being carried out to clarify this important point. While the results reported here do not conclusively establish the identity of the CAT cell as an adipocyte or preadipocyte, they do indicate that the CAT cell differs significantly from the cultured fibroblast in terms of its intracellular levels of cyclic AMP and their response to hormones.

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