Vol. 85, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 14, 1978 Pages 183-189

# COMPARATIVE DEGRADATION OF ADENYLNUCLEOTIDES BY CULTURED ENDOTHELIAL CELLS AND FIBROBLASTS

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Received July 10,1978

SUMMARY

The abilities of cultured human endothelial cells and skin fibroblasts to degrade adenylnucleotides were compared. The cells were incubated, either adherent in the culture dish or in suspension, for 5 min at 37° C with $10^{-5}$M ($^{14}$C) - ATP, -ADP and -AMP and the metabolites in the supernatant were analyzed.

Endothelial cells showed a much greater ability to degrade ATP and ADP while fibroblasts were more efficient to degrade AMP. Due to the small amount of adenosine deaminase activity of endothelial cells, there was an accumulation of adenosine in the medium, while fibroblast suspensions were able to convert a large part of adenosine to inosine. Nucleotide phosphorylation occurred mainly in suspensions of fibroblasts which converted ADP preferentially to ATP. These results suggest a possible contribution of endothelial ADP degradation and of the subsequent adenosine accumulation in the endothelial cell inhibition of platelet aggregation. Differences in the enzymatic activities exhibited by adherent and scraped cells were apparent.

## INTRODUCTION

There is evidence that about 10 times more circulating adenylnucleotides are degraded by the vascular wall than they are by blood. This was shown by Brashear and Ross (1) for ADP clearance in the pulmonary circulation and by Frick and Lowenstein (2) for AMP degradation in the perfused heart. With histochemical methods, nucleotide phosphatase activities have been identified in the capillaries of myocardium (3, 4) and more accurately in the pinocytic vesicles of capillary endothelium (5). Further studies have shown that ADPase activity was present in the membrane fractions prepared from whole aortic homogenate (6) and in aortic rings (7).

Degradation of ADP and subsequent formation of adenosine may play a role in the local control of blood flow and also in the inhibition of

0006-291X/78/0851-0183$01.00/0

platelet aggregation by endothelial cells which was described by Saba and Mason (8). Such nucleotide phosphatases have also been demonstrated in many kinds of cells, either in membrane preparations of hepatocytes (9), fibroblasts (10), platelet (11) or on intact glioma cells (12).

The aim of the present work was to emphasize the ability of endothelial cells to degrade ATP and ADP in comparison with fibroblasts and to explore the different behaviour of monolayer and suspended endothelial cells.

## MATERIAL AND METHODS

### Cell cultures

Human umbilical endothelial cells were cultured according to Jaffe et al (13). Primary subconfluent cultures corresponding to a monolayer of 9 $cm^2$ were used for these experiments.

Human skin fibroblasts were subcultured from the 20th to the 26th passage using the same conditions as for endothelial cells. Subconfluent cultures representing a 9 $cm^2$ area were used.

At subconfluency (5-10 days after plating) the cultures were washed four times with serum free Medium 199 in the culture dish. They were exposed to the adenyl nucleotides either as adherent plaques in the dish or in suspension. Suspensions were obtained by gentle scraping of the cells with a plastic spatula. In the first case, incubations were done in the presence of 1 ml of serum free medium, which covered a 9 $cm^2$ endothelial or fibroblast tissue surface. In the second case, the cells were scraped and collected in one ml of serum free medium. The number of cells was 180 000 - 200 000 as determined after complete dissociation of the cells of replicate control cultures.

### Nucleotide degradation investigations

Adherent cell cultures or scraped cells were exposed to [$^{14}$C] ATP, [$^{14}$C] ADP and [$^{14}$C] AMP, $10^{-5}$ M final concentration, at 37° C in Medium 199 pH 7.4 for 5 min. Then an excess of non labelled adenylnucleotides, 1.6 x $10^{-2}$ M final concentration, was added. Supernatant was rapidly collected and deprived of cells by filtering on Millipore filter (0.2 µ). Nucleotides and metabolites present in the supernatant were analyzed by unidimensional thin layer chromatography on PEI-Cellulose according to Chivot et al (14). Nucleotide separation was achieved by developing the chromatogram in three successive formate solutions at pH 3.5 : 0.5 M, 2 M and 4 M. Nucleosides and purine bases were washed off by methanol. Nucleosides and bases were separated on another chromatogram developed in distilled water.

The spots were identified by autoradiography and then cut out. Their radioactivities were counted in 1 ml distilled water + 10 ml Unisolve. For each component the results were expressed as the percentage of the sum of all spot radioactivities, which represented their relative distribution in the supernatant.

Labelled compounds were purchased from Radiochemical Centre Amersham :

| | | |
|---|---|---|
| 8 - [$^{14}$C] | adenosine 5' monophosphate | 59 mCi/mmol |
| 8 - [$^{14}$C] | adenosine 5' diphosphate | 61 mCi/mmol |
| 8 - [$^{14}$C] | adenosine 5' triphosphate | 53 mCi/mmol |
| 8 - [$^{14}$C] | adenosine | 59 mCi/mmol |
| 8 - [$^{14}$C] | inosine | 57 mCi/mmol |
| 8 - [$^{14}$C] | hypoxanthine | 59 mCi/mmol |
| 8 - [$^{14}$C] | adenine | 61 mCi/mmol |

[$^{14}$C] ATP was contamined with 0.9 % [$^{14}$C] ADP. [$^{14}$C] ADP was contamined with 3.4 % AMP and 1.9 % adenine. [$^{14}$C] AMP was contamined with 2 % adenine. These values were determined by the same chromatographic and autoradiographic procedures as described above.

## RESULTS

### 1) Comparison of adherent endothelial cells and fibroblasts

Comparing adherent fibroblasts and endothelial cells it can be shown (table I) that endothelial cells degraded 58 % of the exogenous ATP mainly in adenosine which represented 72 % of the degradation products. In the same condition fibroblasts degraded only 34 % of the exogenous ATP mainly in adenosine but a small amount of inosine was also detected.

When labelled ADP was used endothelial cells degraded 40 % of the nucleotide, and adenosine represented 67 % of the degraded compounds. Fibroblasts transformed 12 % of the exogenous ADP among which more than half was as adenosine, while 20 % of the metabolite was present as ATP.

When labelled AMP was added to endothelial cells, 57 % was transformed mainly into adenosine and into a very small amount of inosine. Fibroblasts degraded 73 % of the AMP to a large amount of adenosine and some inosine.

It can be noted that, in the various conditions used, the formation of inosine by endothelial cells was not found, except in very small amounts after exposition to labelled AMP.

### 2) Comparison of scraped endothelial cells and fibroblasts

Using scraped fibroblasts or endothelial cells (table II), larger differences among the activities of the two types of cells were found. In the presence of endothelial cells more than 70 % of ATP was degraded, adenosine representing half of the degraded products without any inosine

Table I. Distribution of radioactivities (in percent) of nucleotides and of their metabolites in the supernatant of adherent fibroblasts (F) and endothelial cells (E.C.) exposed to ATP, ADP or AMP $10^{-5}$ M for 5 min at 37° C. 2 experiments were done with fibroblasts and 6 with endothelial cells.

| Labelled compound added | | Supernatant | | | | |
|---|---|---|---|---|---|---|
| | | ATP | ADP | AMP | Adenosine | Inosine |
| ATP | F. | 66.7 | 1.9 | 6.0 | 21.0 | 2.8 |
| | E.C. | 42.0 | 1.3 | 14.0 | 42.0 | |
| ADP | F. | 2.3 | 86.5 | 3.1 | 6.7 | |
| | E.C. | | 60.2 | 12.7 | 27.0 | |
| AMP | F. | | | 27.0 | 67.0 | 5.6 |
| | E.C. | | | 43.0 | 54.5 | 1.5 |

Table II. Distribution of radioactivities (in percent) of nucleotides and of their metabolites in the supernatant of scraped fibroblasts (F) and endothelial cells (E.C.) exposed to ATP, ADP or AMP $10^{-5}$ M for 5 min at 37° C. 3 experiments were done with fibroblasts and 5 with endothelial cells

| Labelled compound added | | Supernatant | | | | |
|---|---|---|---|---|---|---|
| | | ATP | ADP | AMP | Adenosine | Inosine |
| ATP | F. | 71.6 | 13.6 | 5.2 | 4.5 | 4.7 |
| | E.C. | 28.2 | 3.5 | 33.2 | 34.9 | |
| ADP | F. | 30.1 | 51.9 | 6.0 | 6.5 | 5.0 |
| | E.C. | 1.6 | 32.5 | 22.7 | 42.8 | |
| AMP | F. | | | 1.7 | 54.8 | 43.0 |
| | E.C. | | | 48.1 | 50.2 | 1.6 |

formation. In the same condition, fibroblasts apparently converted less than 30 % of ATP, inosine representing about 15 % of the catabolites.

When endothelial cells were exposed to ADP, 68 % of the nucleotide was degraded and the main catabolite was adenosine without any transformation into inosine. Using fibroblasts, half of the exogenous ADP was transformed mainly (2/3) into ATP.

Using labelled AMP the differences were also evident since endothelial cells degraded half of the nucleotide into adenosine while fibroblasts catabolized almost entirely the nucleotide into similar amount of adenosine and inosine.

## DISCUSSION

This investigation was undertaken in order to emphasize the role of endothelial cells in the degradation of adenylnucleotides, especially ADP, which may be involved in the inhibition of platelet aggregation by endothelial cells (8). A comparison was made with fibroblasts, which do not inhibit platelet aggregation (15).

The investigation of enzymatic activities of cultured cells may raise some interpretative problems, related to the maturity of the cultures and to the physical state of the cells. Endothelial cells and fibroblasts are normally organized in a tissue structure. The dissociation of the cells, mechanically or enzymatically, may modify some activities by exposing greater cellular surface, by injuring the cells or by exposing different cellular faces. The modifications of enzymatic activity observed, depended upon the nature of the cells. For endothelial cells, ATP and ADP degradations were increased after scraping. A minimal conversion of ADP to ATP could be also observed. For fibroblasts the scraping induced a marked transformation of ADP into ATP, as well as enhancement of AMP and adenosine degradation. The cell dissociation procedure that is employed may alter the balance among enzymatic activities and must be taken into account when investigating functions of cultured cells.

Whatever the physical state of the cells, cultured endothelial cells exhibited a greater ability to degrade ATP and ADP than did fibroblasts, leading to an accumulation of adenosine while fibroblasts were able to convert it into inosine. These results suggested a low adenosine deaminase activity in endothelial cells. Endothelial cells did not form appreciable amounts of ATP after exposure to ADP while in the same conditions scraped fibroblasts synthetized ATP. Degradation of AMP to adenosine was more significant in the presence of fibroblasts than in presence of endothelial cells. Adenosine deamination was greatest in the presence of scraped fibroblasts. These data are in good agreement with the low purine salvage capacity of fibroblasts exposed to AMP, which has been related to their high 5'nucleotidase and adenosine deaminase enzymatic activities (16).

Until now the antiaggregating activity of endothelial cells has been attributed to the formation of prostacyclin (15, 17, 18) from platelet endoperoxides. However, in a recent report (7), Lewis et al have suggested that the ADPase activity of aortic rings which is potentiated by two cyclocxygenase inhibitors, could be another natural mechanism for limiting thrombotic events. Thus, the degradation of ADP associated with the accumulation of adenosine in the surrounding medium may also contribute to endothelial cell inhibition of platelet aggregation by increasing platelet cyclic AMP (19) or by inhibiting the platelet calcium release as it has been proposed for vascular smooth muscle (20).

Present work shows that we have to take into account that the degradation of ADP by endothelial cells and the subsequent accumulation of adenosine could play a role in the inhibition of platelet aggregation by endothelial cells, since adenosine inhibits ADP-induced platelet aggregation (21).

ACKNOWLEDGEMENTS

We thank Pr. Morin of the Maternity of Lariboisière Hospital for providing the umbilical cords and Carmen Bernard for giving human skin fibroblasts. This work was supported by Grant INSERM, ATP 78-100.

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