PURINE METABOLISM IN MAN—III

Biochemical, Immunological, and Cancer Research

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PURINE METABOLISM IN CULTURED CORONARY ENDOTHELIAL CELLS

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Although it is well known that endothelial cells are involved in several biological processes such as transport[1], hemostasis[2], synthesis of collagen[3], histamine[4] and prostaglandins[5], our knowledge concerning intermediary metabolism of the endothelium is rather limited. In the course of studies on interrelationships between heart function and cardiac metabolism[6,7] we became interested in some features of purine metabolism of coronary endothelial cells. Our interest was initiated by the assumption that these cells might contribute to the production of vasoactive adenosine which is considered to play an important role in the metabolic regulation of coronary blood flow[8,9]. The studies - not possible of course to be performed under in vivo conditions - were carried out on cultured endothelial cells isolated from coronary vessels of guinea pig hearts as recently described[10].

MATERIALS AND METHODS

Culture Medium 199 (Seromed, München) containing penicilline (200 U/ml) and streptomycine (200 µg/ml) was supplemented with fetal calf serum (20%) and L-glutamine (2 mM). Column packings (totally porous silica) for High Pressure Liquid Chromatography (HPLC) were obtained from Macherey & Nagel, Düren. Nucleotides, nucleosides and bases for calibration were purchased from Boehringer Mannheim, all other materials of highest available purity from Merck, Darmstadt.

Preparation of cells and cell culture: Guinea pig hearts were cannulated through the aorta, and their coronary system, washed

free of blood, was filled with an isotonic buffer solution containing collagenase and trypsin (0.1% each). After an exposure of 20 min perfusion was started again and all endothelial cells which had been detached, were collected from the perfusate by centrifugation. Subsequently, the cells were washed with culture medium and seeded in culture dishes. Cultivation was performed at 37°C in a humidified air atmosphere containing 3% CO_2. Depending on the inoculum confluency was reached after 2 to 4 weeks. Contaminations with fibroblasts and smooth muscle cells were usually less than 2%. As judged by electron microscopy the cultivated endothelial cells revealed important morphological criteria of endothelial cells in vivo (clusters of free ribosomes, smooth and rough endoplasmic reticulum, clumps of coarse filaments, fine filaments and prominent microtubules in the cytoplasma).

Analysis of nucleotides, nucleosides and bases: Cultured endothelial cells were extracted with 0.4 N perchloric acid. Quantitation of the different purine compounds in the neutralized cell extracts was carried out by application of specially elaborated HPLC-techniques using weak anion exchange columns for the separation of the nucleotides and reverse phase columns for nucleosides and bases.

Determination of enzyme activities: Specific activities of enzymes involved in nucleotide metabolism were measured in a 20 000 g membrane preparation as well as in a soluble 200 000 g supernatant fraction of endothelial cells. Enzyme tests were performed using standard procedures, substrates and products were separated by HPLC.

RESULTS AND DISCUSSION

In Table 1 mean values from three individual series of analyses concerning contents of purine nucleotides, nucleosides and bases in non-growing confluent endothelial cell cultures are listed. For reasons of comparison respective data for normoxic myocardial tissue are also given. Obviously, endothelial cells contain extraordinarily high amounts of ATP, ADP and AMP. The sum of the adenine nucleotides (ΣATP, ADP, AMP) reaches with more than 15 µmoles/g a value which is about three times higher than the mean adenine nucleotide content of cardiac tissue. Another interesting feature of endothelial cells concerns their high levels of adenine nucleotide degradatives. The contents of adenosine, inosine, adenine and hypoxanthine are about 1 to 2 orders of magnitude higher than the respective values for the myocardium. In contrast to the high levels of adenine nucleotides guanine nucleotides are present in endothelial cells only in small quantities, which are similar to those in myocardial and other tissues.

Table 1: Content of adenine nucleotides and their dephosphorylated degradatives in confluent coronary endothelial cells and in myocardial tissue of guinea pigs. Mean values from three individual series of analyses of 11 culture dishes each.

	Endothelial cells nmoles/g	Myocardium nmoles/g
ATP	11 960	4 280
ADP	2 760	1 050
AMP	630	160
$\sum$ ATP, ADP, AMP	15 350	5 490
Adenosine	87	2
Inosine	100	1.2
Adenine	60	0.5
Hypoxanthine	50	0.9
GTP	233	200
GDP	157	100
GMP	37	23
Guanosine	*)	*)
Guanine	*)	*)

*) not detectable

Additional experiments revealed that growth state of the cultures did not profoundly influence the total content of adenine nucleotides. Furthermore, incubation of confluent cell cultures in purine-free media for three days did not result in any detectable reduction of the adenine nucleotide content. On the other hand, endothelial cells proved to be sensitive to lack of oxygen. It is evident from the data in Fig. 1 that brief periods of anoxic incubation (1.5 and 3 min, respectively) cause a pronounced decrease of ATP with a corresponding increase in ADP and AMP levels. Simultaneously, remarkable amounts of adenosine are formed and released from the cells into the incubation medium.

It appears from all these observations that the extremely high adenine nucleotide levels are very likely a specific feature of cultured endothelial cells. This view is further supported by determinations of activity values of enzymes involved in degradation and synthesis of adenine nucleotides (Table 2). While 5'-nucleotidase activity in endothelial cells exceeds by far that of myocar-

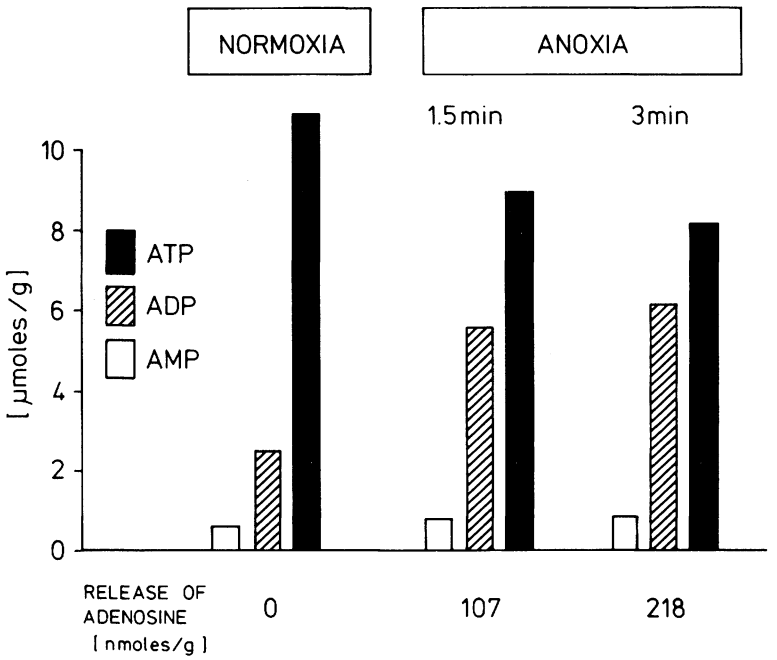

Fig. 1: Influence of anoxia on levels of adenine nucleotides in coronary endothelial cells and on the release of adenosine into the medium (mean values from 3 experiments)

dial tissue, the opposite holds true for adenosine deaminase, the activity of which is much higher in the myocardium. These differences in the pattern of enzyme activities may reasonably explain that endothelial cells contain adenosine in rather high amounts compared with the small quantities of this nucleoside found in the myocardium. As is further evident from the data in Table 2, activities of G-6-PDH and PRPP-synthetase, indirectly involved in the biosynthesis of nucleotides, proved to be much higher in endothelial cells than in cardiac tissue. These findings are in accordance with results from preliminary studies, in which by use of 1-^{14}C-glycine and ^{14}C-labeled purine bases purine nucleotide synthesis in endothelial cells was shown to proceed via salvage and de novo pathways.

SUMMARY

Endothelial cells from coronary vessels of guinea pig hearts were isolated, cultivated and morphologically characterized.- Cells

Table 2: Enzyme activities in cultured coronary endothelial cells and in cardiac tissue from guinea pigs.

	Specific activity [nmoles/min·mg]	
	Endothelial cells	Cardiac tissue
5'-Nucleotidase [E.C. 3.1.3.5]	95	13.6
Alkaline phosphatase [E.C. 3.1.3.1]	14.6	24
AMP deaminase [E.C.3.5.4.6]	1.1	2.2
Adenosine deaminase [E.C. 3.5.4.4]	3.4	28
Glu-6-P-dehydrogenase [E.C. 1.1.1.49]	12.7	4.2
PRPP-synthetase [E.C. 2.7.6.6]	6.58	1.9
APR-transferase [E.C. 2.4.2.7]	0.7	0.2
GPR-transferase [E.C. 2.4.2.8]	0.3	0.1
Adenylate cyclase [E.C. 4.6.1.1]	0.1	0.4
Phosphodiesterase [E.C. 3.1.4.1]	2.1	15

from confluent cultures contained adenine nucleotides and their dephosphorylated degradatives in exceptionally high amounts.- Adenine nucleotide levels were only slightly influenced by the growth state of the cultures and remained stable during incubation for three days in purine-free medium. In contrast, brief incubation of endothelial cells under anoxic conditions resulted in a substantial breakdown of adenine nucleotides associated with an enhanced formation and release of adenosine.- Measurements of specific activi-

ties of enzymes involved in adenine nucleotide synthesis and degradation lend additional support to the view that a very active adenine nucleotide metabolism is a typical feature of cultured coronary endothelial cells.

REFERENCES

1) F. Clementi and G. E. Palade, Intestinal capillaries. Permeability to peroxidase and ferritin, J. Cell. Biol. 41:33 (1969)
2) E. A. Jaffe, L. W. Hoyer, and R. L. Nachman, Synthesis of von Willebrand factor by cultured human endothelial cells, Proc. Natl. Acad. Sci. USA 71:1906 (1974)
3) B. V. Howard, E. J. Macarak, D. Gunsen, and N. A. Kefalides, Characterization of the collagen synthesized by endothelial cells in culture, Proc. Natl. Acad. Sci. USA, 73:2361 (1976)
4) T. M. Hollis and L. A. Rosen, Histidine decarboxylase activity of bovine aortic endothelium and intima-media, Proc. Soc. Exp. Biol. Med. 141:978 (1972)
5) M. A. Gimbrone Jr. and R. W. Alexander, Angiotensin II stimulation of prostaglandin production in cultured human vascular endothelium, Science 189:219 (1975)
6) J. Schrader and E. Gerlach, Compartmentation of cardiac adenine nucleotides and formation of adenosine, Pflügers Arch. 367:129 (1976)
7) J. Schrader, S. Nees, and E. Gerlach, Evidence for a cell surface adenosine receptor on coronary myocytes and atrial muscle cells, Pflügers Arch. 369:251 (1977)
8) R. M. Berne, Cardiac nucleotides in hypoxia: a possible role in regulation of coronary blood flow, Am. J. Physiol. 204:317 (1963)
9) E. Gerlach, B. Deuticke and R. H. Dreisbach, Der Nucleotid-Abbau im Herzmuskel bei Sauerstoffmangel und seine mögliche Bedeutung für die Koronardurchblutung, Naturwissenschaften 50:229 (1963)
10) S. Nees, A. L. Gerbes, B. Willershausen-Zönnchen, and E. Gerlach, Isolation, culture and morphologic characterization of endothelial cells from coronary vessels, abstract of the 51st Meeting (Spring Meeting) of the Deutsche Physiologische Gesellschaft, Kiel (1979)