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Maximum activities of some key enzymes of glycolysis, glutaminolysis, Krebs cycle and fatty acid utilization in bovine pulmonary endothelial cells

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Despite the importance of endothelial cells little is known about their metabolic fuel requirements. To provide some information in this area, the maximum catalytic activities of key enzymes of important metabolic pathways have been measured in bovine pulmonary endothelial cells. The results suggest that both glucose and glutamine are important fuels for these cells: in addition, the oxidation of fatty acids may also be of quantitative significance. The activity of glutaminase in these cells was about 20-fold higher than that in lymphocyte, a cell which exhibits high rates of glutaminolysis. It is suggested that a high rate of glutamine metabolism by endothelial cells is important not only for energy provision but also for provision of nitrogen for biosynthetic purposes including production of local messengers.

Enzyme activity; Glutamine; (Bovine pulmonary epithelial cell)

1. INTRODUCTION

Since endothelial cells occupy strategic positions, sandwiched between blood and parenchymal cells, it is not surprising that they perform important physiological roles such as regulating the rate of blood flow, modulating the activity of neighbouring cells and presenting antigens to specific T-cells. Furthermore, although under normal conditions, cell turnover of endothelial cells from the myocardium of an adult rat is low (about 1% of that observed in epithelial cells from the duodenum) [1], the rate of proliferation can be markedly increased, for example in injury, wound healing, endurance exercise-training in skeletal muscle, corpus luteum formation in the ovary, psoriasis in the skin, diabetic retinopathy and during solid tumour invasion.

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Recently, it has been shown that cells which have the potential for rapid cell division (e.g. lymphocytes, thymocytes) obtain most of their energy from glycolysis (glucose conversion to lactate), glutaminolysis (glutamine conversion to aspartate and lactate) and possibly the complete oxidation of fatty acids [2–4]. Hence, the question arose as to whether endothelial cells obtained their energy in a similar manner. To the authors' knowledge the only systematic metabolic studies on these cells have concerned lipid and cholesterol synthesis from acetate or glucose [5,6].

An indication of the maximum capacity of a given metabolic pathway can be obtained from the maximum in vitro catalytic activity of key enzymes in that pathway [7,8]. This approach has been used in the present study to provide information on metabolism in endothelial cells.

2. MATERIALS AND METHODS

2.1. *Materials*

Tissue culture medium 199 with modified

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Earle's salts, foetal bovine serum, penicillin-streptomycin and gentomycin were obtained from Gibco (Paisley, Scotland).

2.2. *Endothelial cell cultures*

Bovine pulmonary artery endothelial cells were cultured from calf pulmonary arteries as described [9]. The cells were grown in medium 199 with 10% foetal bovine serum replacement, foetal bovine serum (5%, w/v), L-glutamine (2 mM), penicillin-streptomycin (50 U/ml and 50 μg/ml, respectively), gentomycin (20 μg/ml), $NaHCO_3$ (4.35 g/l) and incubated at 37°C in an atmosphere of 5% CO_2 in air. The cells were separated for subculture avoiding the use of proteolytic enzymes. The cells were characterised by contact inhibition of growth and cobblestone appearance in monolayer [10], the presence of factor VIII using indirect immunofluorescence [11] and the presence of angiotensin-converting enzyme activity [12].

2.3. *Preparation of homogenates and enzyme assays*

Homogenates of rat mesenteric lymph nodes were prepared as in [3]. Endothelial cells were homogenised at 0°C in a small ground-glass homogeniser (1 ml capacity) with 5–10 vols extraction medium. The whole homogenate was used for enzyme assays without further treatment, except for the assay of carnitine palmitoyltransferase (EC 2.3.1.21) where it was important to use a mitochondrial preparation [13] of endothelial cells [4]. The extraction medium and assay methods were similar or identical to those described by Ardawi and Newsholme [2]. In all enzyme assays, 0.05% (v/v) Triton X-100 was added to the assay system to complete the extraction of the enzymes [8]. The final volume of the assay mixtures in all cases was 1.0 ml. For all enzymes studied, preliminary experiments established that extraction and assay procedures were such as to produce maximum enzyme activities [8]. Enzymes were measured as described previously (see [2,13] for details) except for the assays of isocitrate dehydrogenase, both NAD^+ (EC 1.1.1.41) and $NADP^+$ (EC 1.1.1.42)-linked, activities which were measured by the method of Alp et al. [14]. All spectrophotometric measurements were carried out with a Gilford recording spectrophotometer (model 240) at 25°C, except for glutaminase (EC 3.5.1.2), which was assayed at 37°C.

All enzyme activities are expressed as nmol substrate utilized/min per mg protein. Protein was assayed as described by Bradford [15].

3. RESULTS AND DISCUSSION

The maximal catalytic activities of a number of key enzymes of some important metabolic pathways of cultured endothelial cells from the pulmonary artery of the cow together with those from the lymphocyte of the rat are listed in table 1. The activities of the enzymes from rat lymphocytes are presented to enable comparison to be

Table 1

Maximum catalytic activities of key enzymes of glycolysis, glutaminolysis, Krebs cycle, pentose-phosphate pathway and of long-chain fatty acid utilization of endothelial cells and lymphocytes

Enzymes	Activities (nmol/min per mg protein)	
	Endothelial cells	Lymphocytes
Glutaminase	634 ± 42.0	39.4
Hexokinase	11.8 ± 0.6	17.3
6-Phosphofructokinase	43.5 ± 0.7	25.7
Pyruvate kinase	225 ± 8.6	403
Lactate dehydrogenase	151 ± 1.6	823
Glucose-6-phosphate dehydrogenase	5.6 ± 0.13	17.5
6-Phosphogluconate dehydrogenase	8.6 ± 0.10	21.1
Citrate synthase	38.9 ± 1.1	63.7
NADP-isocitrate dehydrogenase	11.9 ± 0.3	26.9
NAD-isocitrate dehydrogenase	2.7 ± 0.1	6.3
Malic enzyme	3.7 ± 0.1	3.5
Carnitine palmitoyltransferase	1.4 ± 0.14	0.47
ATP-citrate lyase	1.0 ± 0.09	1.1
Aspartate aminotransferase	86.5 ± 3.3	67.4
Alanine aminotransferase	5.9 ± 0.3	10.3
Glutamate dehydrogenase	154.6 ± 6.5	34.0

Data are expressed as means ± SE of 6 separate determinations

made with a cell that is known to utilise glucose, glutamine and fatty acids at high rates [4] and like the endothelial cell, has the potential for rapid cell division. However, because of species differences, any precise quantitative comparisons must be made with caution. Some activities are remarkably similar between the two types of cells. The activities of the key glycolytic enzymes, hexokinase, 6-phosphofructokinase and pyruvate kinase, are similar although lactate dehydrogenase is more than 5-fold higher in the lymphocyte. This suggests that, as in the lymphocyte, the conversion of glucose to lactate is an important process in endothelial cells. Since the activity of 6-phosphofructokinase is much higher than that of hexokinase in the endothelial cell the use of glycogen as a fuel may be more important in these cells. The activity of carnitine palmitoyltransferase is high in the endothelial cells (almost 3-fold higher than that in lymphocytes – table 1) suggesting that fatty acid oxidation may provide a considerable proportion of the energy for these cells. However, the activity of glutaminase is very high in the endothelial cells: it is almost 20-fold higher than that in the rat lymphocyte, which is considered to possess a high activity [4]. This finding suggests that endothelial cells have a markedly high capacity for glutamine utilization. It is possible that this high glutaminase activity in the endothelial cells used here is due to the presence of glutamine in the culture medium: for example, it might stabilise the activity of the enzyme. However, this seems unlikely, since only physiological concentrations (2 mM) of glutamine were added to the culture medium and this decreased to a lower than physiological level during culture.

Glutamine is known to be an important fuel for rapidly dividing cells (fibroblasts, neoplastic cells) [4], for those with the potential for rapid cell division (lymphocytes) [4] and those with a high protein secretory activity (e.g. enterocytes, macrophages) [13,6]: a hypothesis to account for this high rate of glutamine utilization in such cells has been put forward [16–18]. As in these other cells a high rate of glutamine utilization in endothelial cells may provide optimal metabolic control conditions for cell division (e.g. during tissue repair, angiogenesis and/or for formation of sulphated glucosaminoglycans that are continuously secreted by these cells [19]). Furthermore, a recent discovery is that the relaxant factor released from endothelial cells may be nitric oxide: it would be of interest if the high glutaminase activity provides a mechanism for the constant provision of ammonia for formation of nitric oxide [20]. Indeed, there are reports that endothelial cells in culture can release considerable quantities of ammonia into the culture medium [21,22]. If these cells do utilize glutamine at a high rate, this could interfere in the interpretation of results of experiments in which glutamine release or utilization is studied in intact tissue that contains endothelial cells.

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