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Maximal activities of glutaminase, citrate synthase, hexokinase, phosphofructokinase and lactate dehydrogenase in skin of rats and mice at different ages

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The activities of key glycolytic enzymes are, in general, similar at the three different ages of the animals used in this work (very young, adult and old). Glutaminase is present in skin of both mice and rats, but the activity was much lower in adult animals compared to the very young or the old. It is suggested that this activity is important for the provision of nitrogen for the de novo synthesis of purine and pyrimidine nucleotides during the growth of skin in the young animal and for DNA repair in the old animals; it might be important in the adult skin in response to wound healing.

Glutamine; Wound healing; Aging; (Skin)

1. INTRODUCTION

It has been known for some time that skin contains the necessary complement of enzymes to carry out both glycolysis and the Krebs cycle [1]. However, for reasons that are unclear, the rate of glucose conversion to lactate is about 10-fold greater than that of oxidation [2].

Recent work has demonstrated that cells with the potential for rapid rates of proliferation (e.g. lymphocytes, endothelial cells) have a high capacity not only for glycolysis but also for utilising glutamine [3–6]. These characteristics are present in cells that normally undergo rapid cell division (e.g. tumour cells) [7,8]. Since cells of the skin also possess the capacity to divide rapidly (e.g. during wound repair) it seemed important to investigate the maximum activity of the key enzyme for utilisation of glutamine, glutaminase, in these cells and to compare it with activities of key glycolytic and Krebs cycle enzymes. For this reason, skin from rats and mice has been extracted and the maximum activities of hexokinase, 6-phosphofructokinase, lactate dehydrogenase, citrate synthase and glutaminase have been measured. These activities have been determined in animals at three different ages.

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2. MATERIALS AND METHODS

Animals and biochemicals were obtained from the sources previously described [9,10]. The animals were provided with food and water ad libitum and allowed to age in the departmental animal house. Animals were killed by cervical fracture and the fur rapidly removed from the dorsal skin by shaving. Since only small amounts of skin were available, samples from separate animals were pooled prior to extraction: for 0.28-week-old mice, skin from 25 animals was pooled; for 6–8- and 52-week-old mice, skin from 6–7 animals was pooled; for 0.85-week-old rats, skin from 17 animals was pooled; and for 5–7- or 94–95-week-old animals, skin from 4–6 animals was pooled prior to extraction. Full-thickness skin samples were taken and defatted by scraping. They were then weighed (0.5–0.8 g) and cut into small pieces with scissors at 0°C and homogenised (Polytron homogeniser; PCU-2 at position 3) in 9 vols of extraction medium at 0°C. Extraction media were as follows: 50 mM Tris, 1 mM EDTA (pH 7.4) for extraction of lactate dehydrogenase, citrate synthase and hexokinase; 50 mM Tris, 1 mM EDTA, 5 mM $MgCl_2$ (pH 8.2) for 6-phosphofructokinase; and 150 mM potassium phosphate, 1 mM EDTA and 50 mM Tris (pH 8.6) for glutaminase. Homogenates were allowed to stand on ice before centrifuga-

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tion at 600 × g for 10 min in a refrigerated Beckman centrifuge. The assays of lactate dehydrogenase, citrate synthase, hexokinase and 6-phosphofructokinase were performed at 25°C, with that of glutaminase being carried out at 37°C [2]. The methods were designed to measure maximal enzyme activities in crude extracts of tissues [11].

The results were expressed as μmol/min per g dry wt or per g wet wt. The results were analysed statistically by Student's *t*-test for the difference between group means of animals of various ages.

3. RESULTS AND DISCUSSION

In this work, protein content was measured by the method of Bradford [12] so that results could be presented on the basis of wet wt, dry wt or mg protein. However, the latter results have been omitted because of considerable variability. It is possible that this variability is due to the high content of collagen, only some of which might be extracted into solution and which reacts in the method used for measuring protein concentration. Hence, the activities are presented in table 1 on the basis of wet and dry wt, together with those for lymphocytes for comparison.

In the mouse, the activities of the three glycolytic enzymes are, in general, very similar at the three different ages: both hexokinase and phosphofructokinase activities are somewhat lower at 6–8 weeks, although lactate dehydrogenase is higher. The activities of these enzymes in the skin are lower than those previously reported for lymphocytes but, for both phosphofructokinase and lactate dehydrogenase, the difference is only about 50%. Since the lymphocyte is known to utilise glucose via glycolysis at a high rate [4], this suggests that a high rate of glycolysis is also likely to occur in cells of the skin. Citrate synthase activity is much lower in skin than in lymphocytes, which suggests that the capacity of the Krebs cycle is low in skin.

Of possible importance is the fact that a significant activity of the enzyme glutaminase is present in skin: the activities are similar on a dry wt basis in skin from rat and mouse and, furthermore, the activities at the growing age are very similar to those in the much older animals. Thus, in the rat the activity at 5–7 weeks of age is only about 10% of that at 0.85 weeks and in the mouse only about 5%. Since the activities of glutaminase in the very young and the old are similar to those of hexokinase, it is suggested that the utilisation of glutamine is important in these cells for both energy supply and provision of nitrogen for synthesis of purine and pyrimidine nucleotides. In young skin, this could be required for the purpose of growth. However, this is unlikely to be the case for the skin from old animals. It is possible that, in the latter animals, considerably more DNA repair needs to take place (due to, for example, damage from UV light) and this would require a higher rate of de novo synthesis of purine and pyrimidine nucleotides and hence a higher rate of glutamine utilisation.

The low activity of glutaminase in the adult animal may reflect a decrease in growth rate and a satisfactory covering of hair to prevent UV damage to the skin. It would be of considerable interest to ascertain whether damage to the skin, which would necessitate proliferation of cells for wound healing, would increase the activity of glutaminase at this age.

If it is assumed that the rate of glutamine utilisation by cells is a given proportion of the maximal activity of glutaminase in cells, then it is possible to estimate the rate of utilisation by skin by comparison with lymphocytes [4]. The activity of glutaminase in the very young and in the aged rat is about 20-fold less than that in the lymphocytes but it is likely that there are at least 20-fold more cells in the skin than there are lymphocytes in the rat. Hence, the rate of utilisation of glutamine by skin in these animals could be as high as that in lymphocytes (i.e. about 20 μmol/h per 200 g rat). Since the rate of utilisation by the lymphocytes is considered to be quantitatively significant [5], this calculation suggests that in young and old, and possibly damaged adult skin, the rate of utilisation of glutamine by this tissue may also be significant. In this case, it would be of interest to know whether, in cases such as severe trauma or burns, glutamine availability for the skin and hence rate of glutamine utilisation could limit the rate of skin repair. In this case, topically applied glutamine solutions might improve the rate of wound healing and repair of this tissue: it might be particularly important for burns.

It has been observed that burned skin produces a factor that inhibits the functioning of the immune system [13]. Since cells of the immune system would compete with skin for the limited amount of glutamine produced by the muscle in

Table 1

Maximum activities of hexokinase, 6-phosphofructokinase, lactate dehydrogenase, citrate synthase and glutaminase in skin from young, adult and old rats and mice

Animal	Enzyme	Skin						Lymphocytes (μmol/min per g dry wt)
		Enzyme activity (μmol/min per g fresh wt)			Enzyme activity (μmol/min per g dry wt)			
		(age groups; in weeks)			(age groups; in weeks)			
		0.85	5–7	94–95	0.85	5–7	94–95	
Rat	Hexokinase	0.33 ± 0.07	0.49 ± 0.07[b]	–	1.1 ± 0.25	1.6 ± 0.22[b]	–	7.3
	6-Phosphofructokinase	1.3 ± 0.38	2.46 ± 0.38[b]	0.88 ± 0.22[b]	4.4 ± 1.26	8.2 ± 1.25[b]	2.9 ± 0.75[b]	14.4
	Lactate dehydrogenase	45.2 ± 8.2	85.2 ± 26.1[b]	87.2 ± 16.2[b]	150 ± 27	275 ± 95	283 ± 64[b]	397
	Citrate synthase	1.5 ± 0.22	2.1 ± 0.24[b]	0.67 ± 0.18[b]	4.9 ± 0.72	6.8 ± 0.80[b]	2.2 ± 0.59[b]	41
	Glutaminase	0.38 ± 0.10	0.06 ± 0.02[b]	0.49 ± 0.19[b]	1.9 ± 0.34	0.21 ± 0.07[b]	1.6 ± 0.66[b]	35
		(age groups; in weeks)			(age groups; in weeks)			
		0.28	6–8	52	0.28	6–8	52	
Mouse	Hexokinase	0.63 ± 0.73	0.48 ± 0.12[a]	0.57 ± 0.08	2.1 ± 0.24	1.6 ± 0.39[a]	1.9 ± 0.26	
	6-Phosphofructokinase	1.5 ± 0.24	0.71 ± 0.15[b]	0.93 ± 0.21[b]	5.0 ± 0.81	2.4 ± 0.49[b]	3.1 ± 0.68[b]	
	Lactate dehydrogenase	51 ± 6.5	109 ± 24[b]	80 ± 10.0[b]	170 ± 22	365 ± 80[b]	266 ± 33[b]	
	Citrate synthase	4.7 ± 0.62	1.4 ± 0.20[b]	1.3 ± 0.18[b]	15.7 ± 2.1	4.6 ± 0.65[b]	4.2 ± 0.59[b]	
	Glutaminase	0.60 ± 0.21	0.03 ± 0.02[b]	0.37 ± 0.05[b]	2.0 ± 0.70	0.11 ± 0.57[b]	1.3 ± 0.12[b]	

Results are presented as means ± SE for 6–18 separate pools of skin for rats and 25–38 separate pools of skin for mice. The results obtained for adult or old animals are compared statistically to those from the young animals by Student's *t*-test: [a]$P<0.05$, [b]$P<0.001$. For data for lymphocytes, see [5].

burns, this inhibition of the immune system may be considered to have survival value in conserving an essential fuel for growth and repair of the skin.

This work provides prima facie evidence for the importance of glutamine as a substrate in skin. It does not, however, indicate whether the capacity to utilise glutamine is present in all cells known to be present in skin (e.g. fibroblasts, keratinocytes, hair follicle cells, basal layer cells). The identification of which cells of the skin utilise glutamine is an important problem for the future.

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