The maximum capacity of glycolysis in brown adipose tissue and its relationship to control of the blood glucose concentration

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The maximum activity of the key glycolytic enzymes, hexokinase and 6-phosphofructokinase, was measured in tissues of control and cold-acclimated rats. The only significant change in activity was seen in brown adipose tissue where the activity of these enzymes was increased 2-fold. This increase in glycolytic capacity along with the hypertrophy of BAT observed in cold acclimation suggests that this tissue could play an important role in glucose utilisation by the rat.

Brown adipose tissue *Hexokinase* *Phosphofructokinase* *Blood glucose* *Glycolysis* *Thermogenesis*

1. INTRODUCTION

The control of blood glucose concentration depends on the rates of glucose production and utilisation. Tissues considered to be important in the latter process include the brain, heart, kidney, liver, white adipose tissue and skeletal muscle [1,2] with liver, white adipose tissue and skeletal muscle being able to convert considerable quantities of glucose to the storage fuels, triacylglycerol and/or glycogen. The demonstration that brown adipose tissue (BAT) is capable of high rates of lipogenesis, possibly from glucose [3,4], and that it can receive a high proportion of the cardiac output (up to 35%) [5] raises the possibility that this tissue is quantitatively important not only in thermogenesis [6,7] but also in the utilisation of glucose and hence in the control of the blood glucose concentration. The maximal catalytic activities of hexokinase and 6-phosphofructokinase in muscle provide quantitative indices of the maximum capacities of glycolysis-from-glucose and glycolysis-from-glycogen, respectively [8,9]. The activities of these enzymes have been measured in BAT and other tissues of rats exposed to normal room temperature (22°C) and rats exposed to the cold (4°C) for 14 days (cold-acclimation) and results are presented and discussed here.

2. MATERIALS AND METHODS

Male Wistar rats were obtained from OLAC (1976) Ltd. (Bicester, Oxon). Chemicals and enzymes were from Boehringer Ltd. (Lewes, Sussex) and radiochemicals were obtained from Amersham International (Bucks).

Tissues were removed from the rates ($\sim$ 260 g) and homogenised in an extraction medium containing (mM): 50 Tris, 1 EDTA, 5 $MgCl_2$ and 20 β-mercaptoethanol, at pH 8.2. Hexokinase and 6-phosphofructokinase were assayed spectrophotometrically [10,11] except that hexokinase in brown adipose tissue and hexokinase plus glucokinase in liver were assayed radiochemically [12]. Both these tissues contain a high activity of 6-phosphogluconate dehydrogenase which complicates the usual spectrophotometric assay for these enzymes [13]. Preliminary experiments established that the glucose phosphorylating activity in extracts of BAT was totally inhibited by 0.5 mM glucose 6-phosphate and that there was no increase in activity when the glucose concentration was raised to 50 mM. This indicates that glucose phosphorylation in BAT is catalysed by hexokinase and not glucokinase. For liver, a high concentration of glucose (100 mM) was used to measure the total glucose phosphorylating activity since this tissue

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possesses both hexokinase and glucokinase activities [14].

3. RESULTS AND DISCUSSION

The maximum activities of hexokinase and 6-phosphofructokinase in various tissues of control rats are presented in table 1. The highest activity of hexokinase, on a fresh weight basis, is found in brain but that of BAT is very similar. The highest activities of 6-phosphofructokinase are found in muscle and brain but in these tissues this activity reflects the capacity of glycolysis-from-glycogen rather than glycolysis-from-glucose; the similarity between the activities of hexokinase and 6-phosphofructokinase in both white and brown adipose tissue suggests that glycolysis-from-glucose is the predominant pathway in these tissues. Maintenance of rats at 4°C for 14 days did not change the activity of hexokinase or phosphofructokinase in any tissue except BAT (table 1). For each enzyme, the activity was approximately doubled in this tissue with the result that, after cold-acclimation the highest activity of hexokinase in all tissues investigated (on a fresh wt basis) was that in BAT. In addition, cold acclimation increases the amount of interscapular BAT from 0.21 ± 0.2 g to 0.40 ± 0.3 g ($P < 0.001$). The weight of heart, brain and kidney remained constant but that of the liver decreased from 12.4 ± 0.41 g to 10.3 ± 0.21 g.

It is estimated that interscapular BAT represents ~25% of the total tissue in the rat [5]. Hence it can be calculated that the maximum glycolytic capacity of BAT is ~5.0 and 19.5 μmol/min in control and cold-acclimated animals, respectively, whereas that for total liver is 23.1 and 19.1 μmol/min in control and cold-acclimated animals. Since liver glucokinase in vivo is unlikely to approach saturation with glucose the latter values are probably in excess of in vivo rates so that the capacity of brown adipose tissue for glucose utilisation might be greater than that of the liver in cold-acclimated animals. This suggestion is supported by the finding that the rate of fatty acid synthesis in BAT (one of the possible fates of glucose in this tissue) is 3-fold greater than that in liver in cold-acclimated animals [4]. Alternatively, glycolytically produced pyruvate in BAT could be oxidised via acetyl CoA and the tricarboxylic acid cycle thus providing a fuel for thermogenesis.

Since BAT has a high capacity for glucose utilisation and this glucose could be either oxidised or converted to lipid [15] and since these pathways may be sensitive to insulin [3,17], BAT could play a quantitatively important role in the removal of glucose after a carbohydrate load and hence in the control of blood glucose concentration especially in the cold-acclimated animal. This suggestion is supported by the fact that cold-acclimation increases insulin sensitivity [17]. However, it is

Table 1

The activities of hexokinase and 6-phosphofructokinase in various tissues of control and cold-acclimated rats (μmol . min^{-1} . g fresh $tissue^{-1}$)

Tissue	Hexokinase		6-Phosphofructokinase	
	Control	Cold-acclimated	Control	Cold-acclimated
Brain	8.55 ± 0.22	8.31 ± 0.28	15.43 ± 0.43	14.42 ± 0.74
Heart	5.63 ± 0.15	5.71 ± 0.18	13.40 ± 0.17	13.59 ± 0.61
Liver (glucokinase)	1.87 ± 0.07	1.86 ± 0.14	2.11 ± 0.17	1.98 ± 0.08
Kidney	1.99 ± 0.06	2.02 ± 0.08	3.09 ± 0.11	3.23 ± 0.15
Soleus	1.04 ± 0.08	1.03 ± 0.07	13.01 ± 0.80	10.41 ± 0.50
White quadriceps	0.41 ± 0.02	0.37 ± 0.02	51.42 ± 1.71	56.51 ± 2.24
White adipose	0.28 ± 0.04	0.26 ± 0.03	0.33 ± 0.04	0.45 ± 0.08
Brown adipose	5.99 ± 0.35	12.22 ± 0.73[a]	8.03 ± 0.94	16.83 ± 0.99[a]

Enzyme activities were measured as described in section 2. Activities are presented as the mean ± SEM for 10 different animals and statistical significance (Student's *t*-test) is indicated by [a] $P < 0.001$

not known if the glycolytic capacity of BAT is decreased in conditions of insulin existence (e.g., obesity).

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