Maximal activities of enzymes involved in adenosine metabolism in muscle and adipose tissue of rats under conditions of variations in insulin sensitivity

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The maximal activities of 5′-nucleotidase, adenosine deaminase and adenosine kinase were measured in quadriceps or soleus muscle from animals in which the sensitivity to insulin was changed. Most conditions caused no effect on the activities but exercise-training increased the activity of adenosine deaminase and cold exposure increased the activity of 5′-nucleotidase in soleus muscle: in addition, ageing decreased markedly the activities of all three enzymes in both muscles. When the activities are based on mg protein they are much higher in both white and brown adipose tissue than in muscle, suggesting that changes in adenosine concentration may be important in changing insulin sensitivity in adipose tissue whereas changes in adenosine receptor number may be more important in muscle.

Adenosine *Insulin sensitivity* *Soleus muscle* *Brown adipose tissue* *5′-Nucleotidase* *Adenosine kinase* *Adenosine deaminase*

1. INTRODUCTION

There is now considerable evidence that changes in the sensitivity of muscle glycolysis to insulin, which occur in some physiological or pathological conditions, are due either to changes in the local concentration of adenosine or to a change in the number or affinity of adenosine receptors in the muscle. Thus, increased sensitivity of isolated soleus muscle to insulin caused by exposure of the animal to cold (4°C) is removed by addition of an adenosine agonist (2-chloroadenosine) to the incubation medium, whereas resistance exhibited by the muscle from the genetically obese Zucker rat, or from the rat in which resistance is produced by dietary means, is removed by addition of an adenosine antagonist (8-phenyltheophylline) [1–3].

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A change in adenosine concentration in muscle could be produced by a change in the maximum catalytic activity of one or more of the three enzymes that control the adenosine concentration, that is, 5′-nucleotidase, which produces adenosine from AMP, and adenosine kinase plus adenosine deaminase which remove adenosine [4]. Indeed changes in maximum activity of one or more of these enzymes occur in adipose tissue during conditions in which insulin sensitivity is modified [5]. Insulin sensitivity is increased by starvation, adrenalectomy, physical training or cold exposure whereas the sensitivity is decreased in obesity, by a high carbohydrate diet and by ageing (see section 3). To investigate whether changes in the maximum catalytic activities of these three enzymes play a role in changes in insulin sensitivity in muscle, the activities have been measured in extracts of muscle from such animals. In addition, the activities have been measured, for the first time, in brown adipose tissue, and the activities of these en-

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zymes in white adipose tissue and muscle are presented for comparison.

2. MATERIALS AND METHODS

Rats, chemicals, biochemicals and enzymes were obtained from sources given previously [1,6,7]. Adrenalectomised rats were obtained and were treated prior to sacrifice as in [5]. Obese Zucker rats and lean litter male controls were obtained from OLAC (1976), Blackthorn, Bicester, Oxon, OX6, OTP. Rats were exercised-trained by running on a rodent treadmill as in [8]. Rats were not exercised on the day of sacrifice. For cold exposure, rats were maintained in the department's animal house for one week before being transferred to a cold environment (4°C for two days). This is known to increase the sensitivity of glycolysis in soleus muscle to insulin [1]. Rats were killed by cervical dislocation and quadriceps and/or soleus muscles rapidly dissected and extracted as in [7]. 5′-Nucleotidase, adenosine deaminase and adenosine kinase were assayed as in [5,9].

3. RESULTS AND DISCUSSION

Studies on the effects of adenosine deaminase [10], adenosine receptor agonists and antagonists [6] together with the effect of these agents on muscle removed from animals in which insulin sensitivity is changed [2,3] support the view either that an increase in the concentration of adenosine or an

Table 1

Activities of 5′-nucleotidase, adenosine kinase and adenosine deaminase in muscle from rats in which insulin sensitivity is changed

Muscle	Condition	Enzyme activities (μmol/min per g fresh wt)		
		5′-Nucleotidase	Adenosine kinase	Adenosine deaminase
Quadriceps	Normal, fed 20 days old	71 ± 6.0 (4)	20 ± 5.5	404 ± 52
	Normal, fed 28 days old	57 ± 7.2 (4)	17 ± 1.2	422 ± 31
	Normal, fed 120 days old	44 ± 4.7[a] (4)	8.8 ± 1.1[a]	170 ± 12[a]
Quadriceps	Normal, fed	36 ± 4.3 (5)	11.5 ± 1.4	217 ± 10
	Normal, 48 h starved	41 ± 4.9 (5)	11.5 ± 1.3	212 ± 13
Quadriceps	Sham-operated control	39 ± 3.0 (6)	14 ± 1.0	356 ± 24
	Adrenalectomised	45 ± 3.3 (6)	14 ± 1.2	372 ± 15
Quadriceps	Lean control (*Fa/?*)	58 ± 5.2 (5)	12 ± 0.8	397 ± 17
	Zucker (*fa/fa*) obese	66 ± 4.7 (5)	14 ± 1.0	454 ± 46
Soleus	20 days old	779 ± 113 (5)	8.2 ± 0.79	304 ± 24
	40 days old	726 ± 70 (5)	6.9 ± 0.91	304 ± 25
	150 days old	373 ± 38[a] (5)	4.1 ± 0.21[a]	324 ± 23
Soleus	Sedentary-control	342 ± 41 (5)	12 ± 1.5	439 ± 27
	Exercise-trained	466 ± 48 (5)	12 ± 1.6	549 ± 13[a]
Soleus	Normal, fed	323 ± 43 (5)	13 ± 0.80	622 ± 43
	Starved (48 h)	269 ± 32 (5)	13 ± 0.80	575 ± 16
Soleus	Control	312 ± 90 (6)	24 ± 2.0	991 ± 187
	Cold exposure (48 h)	832 ± 300[a] (8)	25 ± 1.3	760 ± 127
Soleus	Fed	922 ± 37 (4)	8.5 ± 0.56	1081 ± 73
	Starved (48 h)	873 ± 61 (4)	8.6 ± 0.40	1266 ± 49
Heart	Lean control (*Fa/?*)	510 ± 65 (5)	30 ± 2.5 (6)	1157 ± 28 (4)
	Zucker (*fa/fa*) obese	503 ± 70 (5)	32 ± 1.9 (6)	1117 ± 54 (4)

[a] $P < 0.01$

Assays were carried out as described in section 2. Statistical significance was determined by Student's *t*-test. Results are presented as means ± SE with number of separate animals used given in parentheses

increase in the number of adenosine receptors decreases the sensitivity of glycolysis to insulin and vice versa. There is considerable evidence that sensitivity to insulin either in vitro or in vivo is increased by starvation [11], adrenalectomy [12], exercise training [13] and cold exposure [14] and some of these conditions are known to increase the sensitivity of glycolysis to insulin in the isolated soleus muscle [3,8]. Insulin sensitivity in vivo is known to be decreased by genetic obesity [15] and ageing [16], and the isolated soleus muscle from the Zucker genetically obese rat exhibits marked insulin resistance in vitro [2]. The maximum activities of 5′-nucleotidase, adenosine deaminase and adenosine kinase have been measured in quadriceps and soleus muscle from such rats. However, most of these conditions had no significant effect on the activities of these enzymes; these conditions include adrenalectomy, which has profound effects on the activities of these enzymes in white adipose tissue [5], genetic obesity, starvation for 48 h and cold exposure for 48 h in the quadriceps (table 1). Exercise-training caused a small increase in the activity of adenosine deaminase in soleus muscle which would be expected to lower the concentration of adenosine in the muscle and hence improve insulin sensitivity, which is in fact observed. But cold exposure, which also increases insulin sensitivity, increases markedly the activity of 5′-nucleotidase in soleus muscle (table 1) which would be expected to decrease insulin sensitivity. Finally, ageing of the animal dramatically decreased the activities of all three enzymes in both muscles which suggests that the rate of turnover of adenosine in muscle will be decreased with age. If adenosine does indeed play a role in local and acute changes in insulin sensitivity, it suggests that such changes would be more sluggish in the elderly.

It is concluded that, in most if not all conditions investigated, changes in the maximum activities of the enzymes that control the adenosine concentration in muscle cannot be responsible for changes in insulin sensitivity. Although it is possible that changes in adenosine concentration could be achieved by changes in allosteric modulation or covalent modification of these enzymes, we consider that the current results indicate that a change in the number of receptors for adenosine rather than a change in adenosine concentration is responsible for the observed changes in insulin sensitivity.

The activities of these three enzymes were also measured in white and brown adipose tissue and are presented as nmol/min per mg protein for comparison with muscle. The interesting finding is that the activities are much higher in white adipose tissue than muscle, 5′-nucleotidase is about two orders of magnitude higher. This implies that adenosine is important in white adipose tissue and that changes in enzyme activities could produce rapid and marked effects on the adenosine concentration. This is consistent with the previous findings that changes in these activities do occur in white adipose tissue under conditions in which insulin sensitivity changes [5].

Table 2

Activities of 5′-nucleotidase, adenosine kinase and adenosine deaminase in brown and white adipose tissue presented on the basis of protein concentration

Tissue	Condition	Enzyme activities (nmol/min per mg protein)		
		5′-Nucleotidase	Adenosine kinase	Adenosine deaminase
White adipose tissue	Fed	11.4 ± 1.31 (6)	0.27 ± 0.04	18.1 ± 1.81
	Starved (48 h)	13.3 ± 2.00 (6)	0.31 ± 0.03	16.2 ± 3.73
Brown adipose tissue	Fed	3.2 ± 0.35 (6)	0.09 ± 0.01	14.2 ± 0.57
	Starved (48 h)	6.8 ± 0.61 (6)[a]	0.12 ± 0.01[b]	19.7 ± 0.92[a]
Soleus muscle	Fed	1.8 ± 0.35 (6)	0.17 ± 0.012	9.4 ± 1.45
Quadriceps muscle	Fed	0.29 ± 0.04 (6)	0.09 ± 0.005	2.7 ± 0.06

Assays were carried out as described in section 2. Statistical significance was determined by Student's *t*-test ([a] $P < 0.01$, [b] $P < 0.05$). Results are presented as means ± SE with number of separate animals used given in parentheses

High activities were also observed in brown adipose tissue and, moreover, upon starvation for 48 h they were increased close to the values observed in white adipose tissue (table 2). This suggests that adenosine could be important in modifying insulin sensitivity in brown adipose tissue. Since this tissue is believed to be quantitatively important in removal of glucose from the blood after a carbohydrate-rich meal [17], changes in adenosine concentration may be important in improving insulin sensitivity in this tissue during this condition.

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