Reversal of dietary-induced insulin resistance in muscle of the rat by adenosine deaminase and an adenosine-receptor antagonist

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1. Transfer of young rats from a maintenance diet to a breeding diet plus 10% sucrose in the drinking water for 4 weeks caused the development of insulin resistance. 2. Inclusion of the enzyme adenosine deaminase or the adenosine-receptor antagonist 8-phenyltheophylline caused a marked increase in the sensitivity of the soleus-muscle strips isolated from the diet-induced insulin-resistant rats: the concentration of insulin giving 50% of maximum response of glycolysis shifted from 500 to less than 20 μ units/ml.

Impaired glucose tolerance occurs in animals in several pathophysiological conditions. These include genetic obesity (York, 1979), dietary obesity induced by increasing fat (Susini & Lavau, 1978; Grundler & Thenen, 1982; Hissin *et al.*, 1982) or carbohydrate intake (Reaven *et al.*, 1979; Wright *et al.*, 1983), pregnancy (Leturque *et al.*, 1984) and disuse through limb immobilization (Seider *et al.*, 1982). Insulin resistance in specific tissues has been demonstrated in both genetic and dietary models of obesity: isolated adipose tissue and muscle from genetically obese Zucker rats or sucrose-induced insulin-resistant rats exhibit insulin resistance (Vrana & Kazdova, 1970; Olefsky, 1976; Reiser & Hallfrisch, 1977; Cushman *et al.*, 1978; Kemmer *et al.*, 1979; Crettaz *et al.*, 1980).

More recently it has been shown that the sensitivity of glycolysis in isolated soleus muscle to insulin is markedly affected by adenosine: adenosine deaminase (an enzyme that converts adenosine into inosine) or the adenosine-receptor antagonist 8-phenyltheophylline improves the sensitivity of glycolysis to insulin (Espinal *et al.*, 1983; Budohoski *et al.*, 1984). It was therefore of considerable interest to determine whether decreasing the adenosine concentration or blocking the adenosine receptor would improve insulin-sensitivity in muscles isolated from rats made insulin-resistant by dietary manipulation; the results of such a study are presented and discussed here.

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Materials and methods

Male Wistar rats were obtained from Bantin and Kingman, Hull, Yorkshire, U.K. At 30 days of age, rats were divided into two groups; the control group were fed on maintenance diet (rat & mouse No. 1 Maintenance; Special Diet Services, Witham, Essex, U.K.) with free access to water; the second group were fed on a breeding diet (FFG; Dixons, Ware, Herts., U.K.) with free access to water containing 10% (w/v) sucrose.

After 7, 14, 21 and 28 days, animals were starved overnight and subjected to a glucose-tolerance test. Animals were given a subcutaneous dose of glucose (1 g/kg body wt.); blood samples were withdrawn from the tail vein and, after deproteinization with HClO_4 , the samples were assayed for glucose (Bergmeyer *et al.*, 1973).

After 28 days, impaired glucose tolerance was observed in the sucrose-fed rats; at this point animals were killed and soleus-muscle strips were dissected and incubated as described previously (Crettaz *et al.*, 1980; Challiss *et al.*, 1983). The rate of glycolysis was measured by following the rate of lactate formation, and glycogen synthesis was measured by [^{14}C]glucose incorporation into glycogen (see Espinal *et al.*, 1983; Challiss *et al.*, 1983).

Results

At the time of death, animals fed on a maintenance diet weighed 177 ± 3 g ($n = 25$), whereas rats fed on breeding diet plus sucrose weighed 173 ± 8 g ($n = 27$). Nonetheless, from the volume of drinking water consumed, the sucrose

intake of the latter group of animals was approx. 10g/day in the final week of the modified diet.

The results of the 28-day glucose-tolerance test are presented in Fig. 1. Although the fasting glucose concentration is similar in both groups of animals, glucose tolerance is markedly impaired in the sucrose-fed animals.

The effects of insulin on the rates of glycolysis and glycogen synthesis in isolated strips of soleus muscles from control and sucrose-fed animals are presented in Table 1. The basal and maximally insulin-stimulated rates of both glycolysis and glycogen synthesis are not markedly different in both groups of animals. However, the sensitivity of glycolysis to insulin is markedly decreased in the sucrose-fed group of animals, the half-maximal response to insulin occurring at about 110 μ units/ml for control muscles and at about 500 μ units/ml for the sucrose-fed animals, assuming that the rate of lactate formation is maximally stimulated at 10000 μ units/ml (Fig. 2).

The effects of the addition of either adenosine deaminase (0.5 unit/ml) or 8-phenyltheophylline (2 μ M) to the incubation medium containing soleus-muscle strips from sucrose-fed rats are shown in Table 1. Both agents dramatically increased the sensitivity of glycolysis to insulin. However, there was no effect on the process of glycogen synthesis (Table 1). The half-maximal response of glycolysis to insulin was decreased from about 500 to 16 and 8 μ units/ml by adenosine deaminase and 8-phenyltheophylline respectively (Fig. 3). These values are similar to those reported for soleus-muscle

strips obtained from normal animals in the presence of adenosine deaminase (Espinal *et al.*, 1983) and 8-phenyltheophylline (Budohoski *et al.*, 1984).

Fig. 1. Effect of diet on glucose tolerance in animals fed on maintenance diet (●) or breeding diet with access to 10% sucrose in drinking water (□).

Rats received 1g of glucose/kg body wt. subcutaneously at zero time. Blood samples (10 μ l) were withdrawn from the tail vein. Each point is the mean of eight observations, and bars represent S.E.M. The statistical significance of the difference in concentration at each point was determined by using Student's *t* test and is indicated as * P < 0.05, ** P < 0.01.

Table 1. Effect of insulin on rates of lactate and glycogen formation by the stripped soleus-muscle preparation of the rat in the presence of 8-phenyltheophylline or adenosine deaminase

Results are presented as means $\pm$ S.E.M. with the numbers of separate incubations, each containing a single muscle preparation from one animal, in parentheses. Statistical significance was established by Student's *t* test. For comparison of breeding diet + sucrose with maintenance diet, * P < 0.05; for the adenosine deaminase or 8-phenyltheophylline effects in modifying insulin action in muscles from animals fed on breeding diet + sucrose, † P < 0.05, †† P < 0.01.

		Rates ($\mu\text{mol/h}$ per g of tissue)		
		Breeding diet plus sucrose		
Insulin ($\mu\text{units/ml}$)	Maintenance diet	No addition	8-Phenyltheophylline	Adenosine deaminase
(a) Lactate formation				
1	6.26 ± 0.45 (6)	6.10 ± 0.50 (9)	6.19 ± 0.47 (10)	6.87 ± 0.18 (4)
10	6.12 ± 0.50 (6)	6.56 ± 0.55 (9)	11.60 ± 1.52 (10) $\dagger\dagger$	10.24 ± 1.16 (4) $\dagger$
100	9.99 ± 0.88 (6)	7.47 ± 0.30 (9)*	12.64 ± 1.42 (9) $\dagger\dagger$	12.66 ± 1.40 (4) $\dagger\dagger$
1000	14.30 ± 1.04 (6)	10.96 ± 0.50 (9)*	13.90 ± 1.06 (9) $\dagger$	14.19 ± 1.26 (4) $\dagger$
10000	14.00 ± 0.93 (6)	13.61 ± 1.31 (9)	13.80 ± 0.83 (9)	14.16 ± 1.20 (4)
(b) Glycogen formation				
1	0.82 ± 0.20 (5)	0.92 ± 0.11 (11)	0.79 ± 0.09 (9)	1.05 ± 0.14 (4)
10	0.99 ± 0.20 (5)	1.28 ± 0.12 (11)	0.96 ± 0.10 (11)	1.13 ± 0.12 (4)
100	1.83 ± 0.20 (5)	1.93 ± 0.25 (11)	1.51 ± 0.15 (9)	1.83 ± 0.11 (4)
1000	2.97 ± 0.69 (5)	3.14 ± 0.53 (10)	2.68 ± 0.38 (10)	2.59 ± 0.26 (4)
10000	3.29 ± 0.48 (5)	3.75 ± 0.53 (10)	3.22 ± 0.16 (9)	3.65 ± 0.40 (4)

Fig. 2. Effects of insulin on the rates of lactate formation by isolated stripped soleus muscles from rats fed on maintenance diet (●) or breeding diet with free access to 10% sucrose (□)

Rats were maintained on their respective diets for 28 days.

Discussion

Young animals that were allowed access to drinking water containing 10% sucrose for 4 weeks developed glucose intolerance, and the sensitivity of glycolysis of the isolated soleus muscles from these animals to insulin was markedly decreased. The concentration of insulin required to cause a half-maximal stimulation of glycolysis was increased 5-fold by this diet. However, the sensitivity of glycogen synthesis to insulin was unaffected. This differential effect of the diet on the sensitivity of glycolysis and glycogen synthesis to insulin [in contrast with the findings with soleus muscles from obese (*fa/fa*) Zucker rats (Crettaz *et al.*, 1980; Challiss *et al.*, 1984)] suggests that the decrease in insulin sensitivity is due to a post-receptor defect (see Crettaz & Jeanrenaud, 1980). Since the effects of insulin on the rates of membrane transport of glucose and glycogen synthesis are independent (via the recruitment of glucose-transporter units to the plasma membrane and the dephosphorylation of glycogen synthase respectively; Wardzala &

Fig. 3. Effect of adenosine deaminase (▲) and the adenosine-receptor antagonist 8-phenyltheophylline (△) on the effect of insulin on lactate formation by isolated stripped soleus muscles from rats fed on breeding diet with free access to 10% sucrose (□)

Rats were allowed access to 10% sucrose for 28 days before being killed. Results are plotted as increase in lactate production above basal rates (for absolute rates see Table 1). Adenosine deaminase was added to a final concentration of 0.5 unit/ml and 8-phenyltheophylline at 2 μM.

Jeanrenaud, 1981; Cohen, 1982), it is possible to change the sensitivity of these two processes to insulin independently. Possible mechanisms for changing the sensitivity of a given process to a hormone or its secondary messenger have been described by Newsholme (1980).

The decreased insulin-sensitivity of the isolated soleus muscle caused by the sucrose diet was restored to normal values by the addition to the incubation medium of adenosine deaminase, which converts extracellular adenosine into inosine, or 8-phenyltheophylline, which is an adenosine-receptor antagonist. The effects of these agents are identical with those observed with muscles from animals fed on a normal main-

tenance diet (Espinal *et al.*, 1983; Budohoski *et al.*, 1984).

The effect of adenosine deaminase is to decrease the extracellular concentration of adenosine, whereas that of 8-phenyltheophylline is to block the adenosine receptor (which is known to exist on the extracellular side of the plasma membrane of some cells; see Arch & Newsholme, 1978). Since it has been shown that the presence of an adenosine-receptor agonist, 2-chloroadenosine, decreases the sensitivity of glycolysis, but not that of glycogen synthesis, in soleus muscle to insulin (Budohoski *et al.*, 1984), the present results strongly support the view that the decreased sensitivity of glycolysis to insulin in soleus muscle from sucrose-fed animals is due to an increased concentration of adenosine, or to an increase in the affinity or number of receptors for adenosine. Consequently, agents that could either lower the local concentration of adenosine or decrease the binding of adenosine in muscle should increase the sensitivity of glycolysis to insulin and hence improve glucose tolerance in insulin-resistant states.

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