Biochem. J. (1984) **221**, 915–917
Printed in Great Britain

Effects of an adenosine-receptor antagonist on insulin-resistance in soleus muscle from obese Zucker rats

R. A. John CHALLIS, Leszek BUDOHOSKI,* Bronwyn McMANUS and Eric A. NEWSHOLME
Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

(*Received 16 April 1984/Accepted 30 May 1984*)

The decreased sensitivity of glycolysis to insulin seen in isolated soleus muscles from genetically obese Zucker rats was abolished by addition of the adenosine-receptor antagonist 8-phenyltheophylline to the incubation medium; 8-phenyltheophylline had no effect on the sensitivity of glycogen synthesis to insulin. These findings suggest that changes in the sensitivity of glucose utilization by muscles of genetically obese rats may be explained, in part, by a modification in either the concentration of adenosine or the affinity of adenosine receptors in skeletal muscle.

Genetically obese Zucker (*fa/fa*) rats exhibit a decreased sensitivity to insulin (Jeanrenaud, 1979), and this has been linked to the decreased ability of various tissues to bind insulin (Kobayashi & Olefsky, 1978; Le Marchand-Brustel *et al.*, 1978), together with further defects distal to the insulin–receptor interaction (Assimacopoulos-Jeannet & Jeanrenaud, 1976; Crettaz *et al.*, 1980).

It has been known for some time that most cells are able to release adenosine and that within a given tissue it functions as a local hormone or messenger (Arch & Newsholme, 1978). Evidence has been presented that decreasing the adenosine concentration in the incubation medium of an isolated soleus muscle of the rat increased markedly the sensitivity of glycolysis to insulin, i.e. it decreased the concentration of insulin necessary to stimulate glycolysis by 50% (Espinal *et al.*, 1983). This suggested that adenosine decreases insulin-sensitivity. Most, if not all, effects of adenosine are mediated through specific receptors (Londos *et al.*, 1980; Wolff *et al.*, 1981), and a range of adenosine-receptor-specific agonists and antagonists have been synthesized (Bruns, 1981; Daly, 1982). 8-Phenyltheophylline is a well-established receptor antagonist (Griffith *et al.*, 1981), and it has been shown that this compound improves insulin-sensitivity in soleus muscles isolated from normal rats (Budohoski *et al.*, 1984).

An important question is whether 8-phenyltheophylline can improve towards normal the impaired sensitivity to insulin of muscle from genetically obese rats. Hence the effects of 8-phenyltheophylline on the sensitivity of glycolysis and glycogen synthesis to insulin in the stripped soleus muscle preparation isolated from lean (*Fa/?*) and obese (*fa/fa*) Zucker rats were investigated, and the results are presented and discussed below.

Materials and methods

Genetically obese (*fa/fa*) male Zucker rats and their lean littermate controls (*Fa/?*) were purchased from OLAC Ltd., Bicester, Oxon OX6 0TP, U.K., and were maintained in the Department's animal house for 1 week before use. At the time of death, obese animals weighed 235 ± 8 g, whereas lean animals were 178 ± 7 g, and both sets of animals were 8–10 weeks of age.

All chemicals and enzymes were obtained from the sources given previously (Challiss *et al.*, 1983; Budohoski *et al.*, 1984).

Incubations were performed as described by Crettaz *et al.* (1980), with the slight modifications given by Challiss *et al.* (1983); rates of conversion of glucose into lactate and glycogen were measured as described by Espinal *et al.* (1983). As found previously (Espinal *et al.*, 1983; Challiss *et al.*, 1983), the stimulation of glycolysis by insulin was similar whether measured radiochemically or spectrophotometrically. Under the incubation conditions, the proportion of glucose oxidized was small (about 20% of the total glucose metabolism in any experiment), which is similar to previous findings (Crettaz *et al.*, 1980; Espinal *et al.*, 1983).

* Present address: Laboratory of Applied Physiology, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland.

Results and discussion

This study has shown that strips of soleus muscle isolated from 8–10-week-old obese (*fa/fa*) Zucker rats and incubated *in vitro* exhibit a marked resistance to the effect of insulin on glycolysis and glycogen synthesis compared with those from lean (*Fa/?*) littermates (Table 1): this confirms the findings reported by Crettaz *et al.* (1980). The half-maximal stimulation of glycolysis and glycogen synthesis occurred in muscles isolated from obese animals at an insulin concentration of approx. 1500 μunits/ml, compared with approx. 150 μ-units/ml for muscles from lean littermates (Table 1 and Figs. 1 and 2).

The effects of 8-phenyltheophylline (2 μM) on rates of lactate and glycogen formation in soleus muscle preparations from lean and obese Zucker rats are given in Table 1. As reported by Budohoski *et al.* (1984), 8-phenyltheophylline caused an increase in the sensitivity of glycolysis to insulin in soleus muscles from control animals. However, the important observation in the present work is that the marked decrease in sensitivity of glycolysis to insulin in muscles from obese animals is abolished by addition of 8-phenyltheophylline to the incubation medium. At a concentration of 2 μM, this adenosine-receptor antagonist decreased the concentration of insulin required to cause a half-maximal increase in the rate of glycolysis from approx. 1500 to approx. 50 μunits/ml (Table 1 and Fig. 1). In contrast, 8-phenyltheophylline had no effect on the sensitivity of the process of glycogen synthesis to insulin (Table 1 and Fig. 2). However, 8-phenyltheophylline decreased rates of glycogen synthesis in the presence of low concentrations of insulin in muscles obtained from both lean and obese animals (Table 1). The mechanism for this is not known, but it is not due to an increase in the rate of glycogenolysis, since there was no difference between spectrophotometrically and radiochemically determined rates of lactate formation

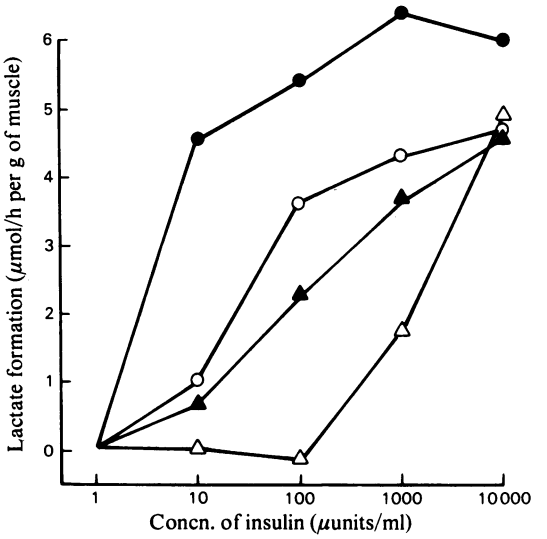

Fig. 1. *Effect of insulin on the rate of lactate formation by incubated stripped soleus muscle from lean (▲ and ●) and obese (△ and ○) Zucker rats incubated in the presence (● and ○) or absence (▲ and △) of 8-phenyltheophylline*

Rates are presented as the increase above that observed above 1 μunit of insulin/ml.

Table 1. *Effects of 8-phenyltheophylline (2 μM) on the rates of lactate formation and glycogen synthesis at different concentrations of insulin in stripped soleus-muscle preparations from lean control (Fa/?) and obese (fa/fa) Zucker rats*

The methods for measuring rates of lactate formation and glycogen synthesis are given in the Materials and methods section. Results are presented as means ± S.E.M. for at least six separate experiments involving single muscle strips. Statistically significant differences (Student's *t* test; $P < 0.05$) for lean rats versus lean rats plus 8-phenyltheophylline are indicated by [a], for obese rats versus obese plus 8-phenyltheophylline by [b] and for lean versus obese by [c].

Concn. of insulin (μunits/ml)	Lean control	Lean control plus 8-phenyltheophylline	Obese	Obese plus 8-phenyltheophylline
	Lactate formation (μmol of lactate/h per g)			
1	8.18 ± 0.82	6.08 ± 0.42[a]	8.33 ± 0.28	8.13 ± 0.50
10	8.82 ± 0.26	10.62 ± 0.46[a]	8.35 ± 0.29	9.10 ± 0.41
100	10.40 ± 0.67	11.46 ± 0.52	8.18 ± 0.75[c]	11.73 ± 1.08[b]
1000	11.83 ± 0.41	12.46 ± 0.27	10.04 ± 0.46[c]	12.40 ± 0.96[b]
10000	12.76 ± 0.82	12.07 ± 1.32	13.20 ± 1.10	12.80 ± 1.18
	Glycogen formation (μmol of glucosyl equiv./h per g)			
1	1.23 ± 0.14	0.94 ± 0.13	1.16 ± 0.04	0.77 ± 0.06[b]
10	1.74 ± 0.14	1.14 ± 0.09[a]	1.09 ± 0.32[c]	0.85 ± 0.11
100	3.00 ± 0.23	2.40 ± 0.56	1.84 ± 0.26[c]	0.72 ± 0.15[b]
1000	3.76 ± 0.37	3.66 ± 0.37	2.44 ± 0.14[c]	1.98 ± 0.31
10000	4.33 ± 0.44	3.97 ± 0.41	3.84 ± 0.70	3.60 ± 0.33

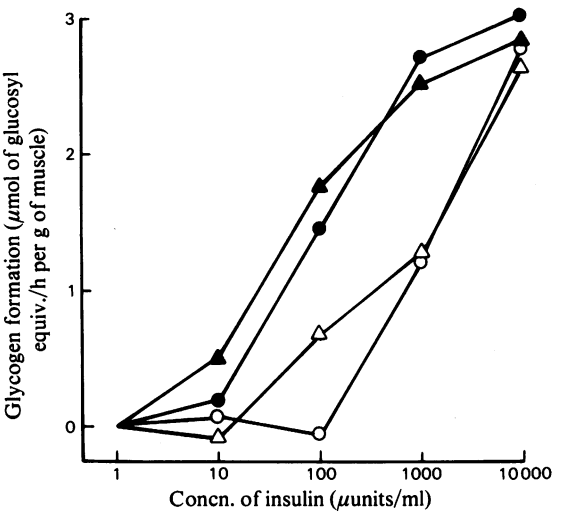

Fig. 2. *Effect of insulin on the rate of glycogen formation by incubated stripped soleus muscle from lean (▲ and ●) and obese (△ and ○) Zucker rats incubated in the presence (● and ○) or absence (▲ and △) of 8-phenyltheophylline*
Rates are presented as that observed above 1 μunit of insulin/ml.

(which measure glucose-plus-glycogen and glucose fluxes to lactate respectively).

Crettaz *et al.* (1980) have shown that in muscles from obese animals there is a 30% decrease in the number of insulin receptors in comparison with the muscles of lean littermates. Since only about 20% occupancy of these receptors in normal muscle is necessary to elicit a maximal response to insulin, Crettaz *et al.* (1980) concluded that the decrease in insulin-receptor number in obesity could not be solely responsible for the observed insulin-resistance. This conclusion is supported by the findings that 8-phenyltheophylline improves the sensitivity of glycolysis, but not that of glycogen synthesis to insulin, although stimulation of both processes presumably requires the binding of insulin to its receptor. A change in sensitivity of the process of glucose transport without a corresponding effect on glycogen synthesis is possible because insulin affects these processes in different ways: transport is increased by an increase in the number of glucose-transporter proteins in the cell membrane (Cushman & Wardzala, 1980; Wardzala & Jeanrenaud, 1981), whereas glycogen synthesis is affected via a covalent modification of glycogen synthase (Cohen, 1982). Furthermore, the stimulation of the rate of glycogen synthesis by insulin in muscle can be metabolically independent of the effect of insulin on glucose transport, since glycogen synthase catalyses a flux-generating step (Newsholme & Leech, 1983).

These findings demonstrate, for the first time, that changes in the sensitivity of glycolysis to insulin in soleus muscle *in vitro*, brought about by a change in the pathological status of the animal, can be normalized by the presence of an adenosine-receptor antagonist. Consequently, insulin-resistance in muscles of the obese animal may be caused, at least in part, by an increase in the concentration of adenosine or by an increase in the affinity of the receptor for adenosine.

We thank Professor R. R. Porter, F.R.S., for his interest and encouragement, and the British Diabetic Association for financial support. L. B. was the recipient of a Fellowship from the Wellcome Trust.

References

Arch, J. R. S. & Newsholme, E. A. (1978) *Essays Biochem.* **14**, 82–123
Assimacopoulos-Jeannet, F. & Jeanrenaud, B. (1976) *Clin. Endocrinol. Metab.* **5**, 337–365
Bruns, R. F. (1981) *Biochem. Pharmacol.* **30**, 325–333
Budohoski, L., Challiss, R. A. J., McManus, B. & Newsholme, E. A. (1984) *FEBS Lett.* **167**, 1–4
Challiss, R. A. J., Espinal, J. & Newsholme, E. A. (1983) *Biosci. Rep.* **3**, 675–679
Cohen, P. (1982) *Nature (London)* **296**, 613–620
Crettaz, M., Prentki, M., Zaninetti, D. & Jeanrenaud, B. (1980) *Biochem. J.* **186**, 525–534
Cushman, S. W. & Wardzala, L. J. (1980) *J. Biol. Chem.* **255**, 4758–4762
Daly, J. W. (1982) *J. Med. Chem.* **25**, 197–207
Espinal, J., Challiss, R. A. J. & Newsholme, E. A. (1983) *FEBS Lett.* **158**, 103–106
Griffith, S. G., Meghji, P., Moody, C. J. & Burnstock, G. (1981) *Eur. J. Pharmacol.* **75**, 61–64
Jeanrenaud, B. (1979) *Diabetologia* **17**, 133–138
Kobayashi, M. & Olefsky, J. M. (1978) *Am. J. Physiol.* **235**, E53–E62
Le Marchand-Brustel, Y., Jeanrenaud, B. & Freychet, P. (1978) *Am. J. Physiol.* **234**, E348–E358
Londos, C., Cooper, D. M. F. & Wolff, J. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2551–2554
Newsholme, E. A. & Leech, A. R. (1983) *Biochemistry for the Medical Sciences*, pp. 583–591, John Wiley and Sons, New York and London
Wardzala, L. T. & Jeanrenaud, B. (1981) *J. Biol. Chem.* **256**, 7090–7093
Wolff, J., Londos, C. & Cooper, D. M. F. (1981) *Adv. Cyclic Nucleotide Res.* **14**, 199–214