Effects of analogues of adenosine and methyl xanthines on insulin sensitivity in soleus muscle of the rat

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The concentration of insulin that produces half-maximal stimulation of glycolysis by stripped soleus muscle preparations is markedly increased by the adenosine analogues, 2-chloroadenosine and N^6-phenylisopropyladenosine, but is markedly decreased by the methyl xanthine analogue, 8-phenyltheophylline. 2-Chloroadenosine increases the concentration of insulin required to stimulate glycolysis half maximally, from about 100 to 2000 μunits/ml. 8-Phenyltheophylline decreases this concentration of insulin from about 100 to 10 μunits/ml, an effect which is similar to that produced either by addition of adenosine deaminase to the medium or to exercise-training of the donor animals for 4 weeks.

Glycolysis *Insulin sensitivity* *Insulin resistance* *Glycogen synthesis*
N^6-Phenylisopropyladenosine *2-Chloroadenosine* *Isobutylmethyl xanthine* *8-Phenyltheophylline*

1. INTRODUCTION

Insulin stimulates the rates of glycolysis and glycogen synthesis in muscle [1]. Evidence has been obtained that adenosine, which is produced endogenously in muscle, decreases the sensitivity of glycolysis to insulin, but does not change the sensitivity of the process of glycogen synthesis. Thus, the presence of adenosine deaminase (ADA) in the incubation medium (which decreases the concentration of adenosine) decreased the concentration of insulin required to produce half-maximal stimulation of glycolysis in isolated soleus muscle from ~100 to 10 μunits/ml [2]. The effect of ADA was abolished by the addition of the adenosine analogue, N^6-phenylisopropyladenosine (PIA). Adenosine has many functions as a local messenger in different tissues [3,4] and most, if not all, of these effects are mediated by binding to an extracellular receptor known as the 'R-site' [5,6]. It is suggested that analogues of adenosine (e.g., PIA) act as adenosine agonists for this site whereas methyl xanthine analogues (e.g., 8-phenyltheophylline (PTh)) act as antagonists [7,8]. If adenosine influences insulin sensitivity through this receptor, it is predicted that adenosine analogues should decrease insulin sensitivity of muscle glycolysis to insulin whereas methyl xanthine analogues should increase this sensitivity. The effects of two adenosine analogues, 2-chloroadenosine (2ClAdo) and PIA, and two methyl xanthine analogues, isobutylmethylxanthine (MIX) and PTh, on the sensitivity of glycolysis, glycogen synthesis and glucose oxidation to insulin in the isolated stripped soleus muscle preparation have been investigated and the results are presented and discussed here.

2. MATERIALS AND METHODS

Animals, chemicals and enzymes were obtained from the sources in [9], except for [U-^{14}C]glucose,

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which was obtained from the Radiochemical Centre (Amersham), PIA which was obtained from Boehringer (Lewes, E. Sussex) and 2ClAdo, MIX and PTh which were obtained from Sigma (London).

Rats were killed by cervical dislocation and the soleus muscle from each leg carefully exposed. The soleus muscle was divided into two strips of 25–35 mg as in [9,10] and attached to stainless steel clips and transferred directly to 4 ml Krebs-Ringer bicarbonate buffer at pH 7.4, containing 1% de-fatted albumin (which had been dialysed overnight against the buffer) and 5 mM glucose, in 25 ml siliconised Erlenmeyer flasks at 37°C (see [10] for details). The buffer had been pregassed with O_2/CO_2 (95:5) for 30 min. The strips were pre-incubated for 30 min, transferred to another flask containing the same medium except for

Table 1

Effect of insulin on the rates of glycogen, lactate and carbon dioxide formation by the stripped soleus muscle preparation of the rat in the presence of analogues of adenosine or methyl xanthines

Insulin concentration (μunits/ml)	Additions to incubation	Rates of formation (μmol·g^{-1}·h^{-1})		
		Glycogen[a]	Lactate[b]	Carbon dioxide[a]
1	None	1.08 ± 0.10	5.53 ± 0.49	0.45 ± 0.20
10		1.22 ± 0.09	6.29 ± 0.39	0.35 ± 0.09
100		2.56 ± 0.24	8.73 ± 0.78	0.51 ± 0.15
1000		3.33 ± 0.37	11.60 ± 0.78	0.82 ± 0.22
10000		2.98 ± 0.08	11.43 ± 0.07	0.69 ± 0.29
1	2-Chloroadenosine	1.25 ± 0.11	6.02 ± 1.30	0.36 ± 0.07
10		1.24 ± 0.09	6.60 ± 0.60	0.37 ± 0.50
100		1.40 ± 0.08	7.65 ± 0.87	0.36 ± 0.10
1000		4.35 ± 0.22	8.10 ± 0.53	0.48 ± 0.08
10000		5.81 ± 0.46	14.0 ± 1.30	1.01 ± 0.08
1	N^6-Phenylisopropyladenosine	0.38 ± 0.04	5.20 ± 0.35	0.79 ± 0.09
10		0.42 ± 0.04	4.80 ± 0.35	0.61 ± 0.07
100		0.58 ± 0.09	5.60 ± 0.41	0.76 ± 0.05
1000		0.75 ± 0.07	6.50 ± 0.30	1.16 ± 0.07
10000		1.04 ± 0.06	9.90 ± 0.95	1.63 ± 0.014
1	Isobutylmethylxanthine	0.98 ± 0.12	7.76 ± 0.32	–
10		1.09 ± 0.13	8.39 ± 0.36	–
100		1.85 ± 0.56	9.58 ± 0.35	–
1000		2.63 ± 0.42	11.48 ± 0.52	–
10000		1.92 + 0.53	12.64 ± 0.60	–
1	8-Phenyltheophylline	0.97 ± 0.13	4.87 ± 0.50	–
10		1.01 ± 0.26	7.43 ± 0.70	–
100		2.16 ± 0.17	9.02 ± 0.60	–
1000		4.47 ± 0.90	10.23 ± 0.52	–
10000		4.15 ± 0.24	9.36 ± 0.32	–

[a] Rates of formation expressed as μmol glucosyl equiv.·g^{-1}·h^{-1}

[b] Rates of formation expressed as μmol lactate·g^{-1}·h^{-1}

Results are presented as means ± SE of at least 6 incubations each containing a single muscle preparation from one animal. Concentrations of adenosine analogues used were: 2-chloroadenosine, 20 μM; phenylisopropyladenosine, 10 μM; isobutylmethylxanthine, 25 μM; 8-phenyltheophylline, 2 μM

the presence of 5 mM glucose containing [U-^{14}C]glucose (0.25 μCi/ml) and insulin at 0–10 munits/ml (see table and figs for details) and incubated for a further 60 min. The flasks were gassed continuously during the preincubation and also for the first 15 min of the incubation period. The incubation was terminated, the muscle and incubation medium treated and the rate of lactate formation, glycogen synthesis and carbon dioxide production measured as in [2].

3. RESULTS

The maximal stimulations of the rates of glycogen synthesis, glycolysis and glucose oxidation by insulin were ~3–4-fold, ~2–3-fold and ~2-fold, respectively. The rate of glucose oxidation was in general about 10% that of glycolysis so that changes in the rate of the former process could not interfere with conclusions concerning the effects of insulin on glycolysis.

The effects of insulin in the presence of analogues of adenosine and methyl xanthine are presented in table 1. Of the analogues tested, 2ClAdo and PIA decreased the sensitivity of the rate of glycolysis to insulin; in the control incubation, a statistically significant increase in the rate of glycolysis was observed at 100 μunits/ml whereas this was only observed at 1000 μunits/ml in the presence of these compounds. In contrast, PTh increased the sensitivity of the rate of glycolysis to insulin; a statistically significant increase in the rate of glycolysis was observed at 10 μunits/ml. The other methyl xanthine analogue, MIX, had little or no effect on the sensitivity of glycolysis to insulin (table 1). The contrasting effects of 2ClAdo and PTh on the rates of lactate formation are presented graphically (fig.1). From the plots, the concentrations of insulin that caused a half-maximal increase in the rate of glycolysis are ~100, ~10 and ~2000 μunits/ml for control, PTh and 2ClAdo incubations, respectively.

The effects of the analogues of adenosine and methyl xanthine on the rates of glycogen synthesis and carbon dioxide formation are much less clear-cut (table 1; fig.2): at 100 μunits/ml insulin,

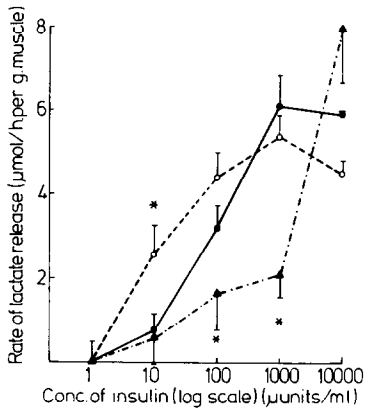

Fig.1. Effect of various concentrations of insulin on the rates of lactate formation by isolated stripped soleus muscle of the rat in the presence of 2-chloroadenosine or 8-phenyltheophylline. Rates of lactate formation are presented as the increment in rate caused by insulin. (●——●) No addition to incubation medium; (▲ - - - - ▲) 20 μM 2-chloroadenosine present in the incubation medium; (○---○) 2 μM 8-phenyltheophylline present in the incubation medium. Differences from the control that are statistically significant (Student's t-test) are indicated by * ($p < 0.05$).

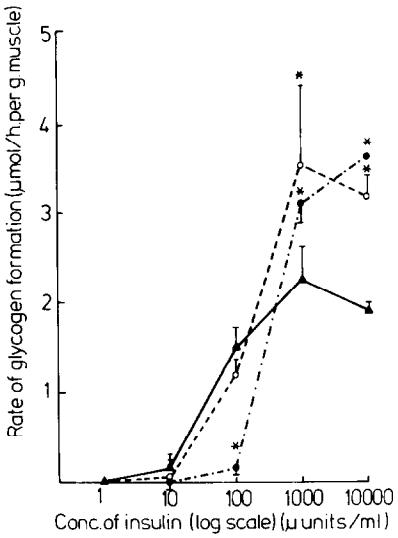

Fig.2. Effect of various concentrations of insulin on the rates of glycogen formation by isolated stripped soleus muscle of the rat in the presence of 2-chloroadenosine or 8-phenyltheophylline. Rates of glycogen formation are presented as the increment in rate caused by insulin. (▲——▲) No addition to incubation medium; (●-----●) 20 μM 2-chloroadenine present in the incubation medium; (○---○) 2 μM 8-phenyltheophylline present in the incubation medium. Differences from the control that are statistically significant (Student's t-test) are indicated by * ($p < 0.05$).

2ClAdo markedly decreased the rate of glycogen synthesis; 2ClAdo and PTh increased whereas PIA and MIX decreased the maximum response of glycogen synthesis to insulin. The analogues had no marked or consistent effect on the response of carbon dioxide formation to insulin concentration (table 1).

4. DISCUSSION

Both analogues of adenosine tested here decreased the sensitivity of the rate of glycolysis to insulin. Indeed, in the presence of 2ClAdo, the concentration of insulin required to stimulate glycolysis half-maximally was increased about 20-fold. It is expected that such an analogue in vivo would produce marked resistance of muscle to insulin. If this represents the normal response of muscle to a high concentration of adenosine, it demonstrates the potential within the muscle for acutely modifying its response to insulin and suggests that the insulin resistance observed in conditions such as obesity, type II diabetes mellitus, pregnancy or lactation may be due, at least in part, either to an increase in the concentration of adenosine in the muscle or to an increase in the number or affinity of adenosine receptors in this tissue. Of the two methyl xanthine analogues tested, only PTh increased the sensitivity of the rate of glycolysis to insulin; the concentration of insulin required to stimulate the rate of glycolysis half-maximally was decreased ~10-fold. This is consistent with the analogue behaving as adenosine antagonist. This is supported by the finding that the effect of PTh is similar in magnitude to that produced by the addition of ADA to the incubation medium [2]. A period of exercise-training (of the donor animal) for 4 weeks improved the sensitivity of glycolysis to insulin in the isolated stripped soleus muscle preparation [9] to a similar extent as that reported here by the simple procedure of adding PTh to the incubation medium. This suggests that exercise-training and indeed other conditions that improve insulin sensitivity in vivo may do so either by causing a decrease in the concentration of adenosine in muscle or by decreasing the number or affinity of adenosine receptors.

The findings presented here suggest a new role for adenosine in muscle – the acute modification of insulin sensitivity. Thus, changes in the concentration of adenosine in muscle may be partly responsible for changes in insulin sensitivity in vivo and this suggests that modification of the activities of the enzymes that control the concentration of adenosine in muscle [11] or administration of muscle-specific adenosine analogues may improve insulin sensitivity in patients suffering from insulin resistance.

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