Effect of adenosine deaminase and an adenosine analogue on insulin sensitivity in soleus muscle of the rat

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The concentration of insulin that produces half-maximal stimulation of glycolysis in stripped soleus muscle preparations is decreased from ~100–10 μunits/ml by the presence of adenosine deaminase in the incubation medium. This suggests that adenosine decreases insulin sensitivity. The effect of the deaminase is abolished by addition of the adenosine analogue, N^6-phenylisopropyladenosine which is not metabolised by the deaminase. The effect of the deaminase in isolated soleus muscle is similar to that of a period of physical training of the rat.

Glycolysis *Blood glucose* *Adenosine deaminase* *N^6-phenylisopropyladenosine* *Insulin sensitivity* *Rat soleus muscle*

1. INTRODUCTION

Insulin stimulates the rates of glycolysis and glycogen synthesis in muscle, and exercise-training of the rat increases the sensitivity of the process of glycolysis of isolated muscle preparations to insulin [1,2]. Adenosine is known to change insulin sensitivity in adipose tissue [3]. Therefore, it was important to investigate whether adenosine could change the sensitivity of glycolysis and glycogen synthesis in muscle to the effects of insulin.

Adenosine is continuously produced by adipose tissue and probably also muscle (review [4]) so that within a few minutes of incubation sufficient adenosine may be produced to obscure the effect of exogenous adenosine. For this reason, and since adenosine is known to be released from a tissue into the interstitial space, the effect of adenosine on insulin sensitivity has been investigated by adding the enzyme adenosine deaminase, which converts adenosine to the inactive metabolite inosine, to the incubation medium.

Effects of adenosine could then be investigated by use of an adenosine analogue that binds to adenosine receptors but is not metabolised by adenosine deaminase. Such an analogue may be N^6-phenylisopropyladenosine. The effects of insulin, adenosine deaminase and the adenosine analogue, N^6-phenylisopropyladenosine, on both the rate of glycolysis and glycogen synthesis in the isolated stripped soleus muscle preparation of the rat have been investigated.

2. MATERIALS AND METHODS

Animals, chemicals and enzymes were obtained from the sources given in [3] except for [U-^{14}C]glucose, which was obtained from the Radiochemical Centre (Amersham).

Rats were killed by cervical dislocation and the soleus muscle from each leg carefully exposed. The soleus muscle was divided into 2 strips of 25–35 mg, were then attached to stainless steel clips and transferred directly to 4 ml Krebs–Ringer bicarbonate buffer at pH 7.4, containing 1% (w/v) de-fatted albumin (which had been dialysed overnight against the buffer) and 5 mM glucose, in a 25 ml siliconised Erlenmeyer flask at 37°C (see [5] for details of method). The buffer had been pre-gassed with O_2/CO_2 (95 : 5) for 30 min. The

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strips were pre-incubated for 30 min, transferred to another flask containing the same medium except for the presence of 5 mM glucose containing [^{14}C]glucose (0.25 μCi/ml) and insulin at 0–10 munits/ml (see tables for details) and incubated for a further 60 min. The flasks were gassed continuously during the preincubation and also for the first 5 min of the second incubation. At the end of the incubation the muscle strips were removed and immediately freeze-clamped. To a volume of incubation medium sufficient 40% $HClO_4$ (w/v) was added to give a final concentration of 4% (w/v) and the protein removed by centrifugation: the supernatant was neutralised with KOH and precipitated $KClO_4$ removed by centrifugation. The supernatant was used for the assay of lactate enzymatically [6] and radiochemically by separation of lactate on an ion-exchange column [7]. The radioactivity incorporated into glycogen was used to measure the rate of glycogen synthesis [8]. The freeze-clamped muscle was extracted in 0.5 ml 1 N NaOH for 1 h: 1.5 ml 75% (v/v) ethanol plus carrier glycogen and Na_2SO_4 was added to the extract and left overnight; the precipitated glycogen was redissolved in 0.5 ml water and reprecipitated and was then dissolved in water and a sample added to 10 ml scintillant which contained 2.0 g 2,5-diphenylixazole and 0.05 g 1,4-bis-(5-phenyloxoazolyl)-2-benzene in 500 ml toluene plus 250 ml Triton X-100 and the radioactivity measured in a liquid scintillation counter (Beckman Model LS 7500). The rate of glucose oxidation was measured by absorption of carbon dioxide in 2 N NaOH after acidification of the medium with 5 N H_2SO_4 and the radioactivity was measured on barium acetate saturated discs [9] as described above.

3. RESULTS

With such small amounts of muscle and 5 mM glucose in the incubation medium it was not possible to measure accurately the decrement in glucose concentration at the end of the incubation. Consequently, the rate of glycolysis was measured by following the formation of lactate; the direct conversion of glucose to lactate was measured by separating lactate and measuring the incorporation of ^{14}C from [^{14}C]glucose [2], the results from the two measurements were similar. The rate of glucose oxidation was <20% of that of glycolysis at zero insulin and <10% at 10000 μunits/ml insulin. The effects of varying concentrations of insulin on the rates of glycolysis in the absence and presence of adenosine deaminase are shown in table 1. In the control incubation, no statistically significant effect of insulin was observed until 100 μunits/ml but a marked stimulation was observed at 10 μunits/ml insulin in the presence of the deaminase (table 1). A plot of the percentage of the maximal increase in rats caused by insulin against the insulin concentration demonstrates that the concentration of insulin which caused half-maximal stimulation was about 100 μunits/ml, in agreement with previous findings, but in the presence of the deaminase the insulin concentration that caused half-maximal stimulation was about 10 μunits/ml (not shown). To demonstrate that this effect of the deaminase was due to adenosine removal, the adenosine analogue N^6-phenylisopropyladenosine was included. Preliminary experiments established that this compound was not metabolised by the deaminase. The analogue prevented the effect of the deaminase (table 1). However, at maximal concentrations of insulin,

Table 1

Effects of insulin on the rates of lactate formation by the stripped soleus muscle preparation of the rat in the presence of adenosine deaminase or adenosine deaminase plus N^6-phenylisopropyladenosine

	Lactate formation (μmol . g^{-1} . h^{-1})		
Insulin (μunits/ml)	No addition	Adenosine deaminase	Adenosine deaminase plus PIA
0	8.08 ± 0.75	7.95 ± 0.51	8.10 ± 0.57
1	8.23 ± 0.90	7.49 ± 0.36[b]	8.34 ± 0.66
10	9.59 ± 1.02	17.31 ± 2.28	9.85 ± 1.09
100	14.93 ± 1.59	18.09 ± 1.94	15.71 ± 1.06
1000	18.47 ± 1.18	17.76 ± 1.66	20.18 ± 3.40
10000	18.16 ± 1.18	19.67 ± 1.84	23.08 ± 1.89[a]

Results are presented as means ±SEM for 6 incubations each containing a single muscle preparation from one animal. The rates of glucose utilisation at 0, 100 and 1000 μunits/ml insulin were 1.24 ± 0.14, 2.56 ± 0.09 and 1.59 ± 0.10, respectively. Differences from the control that are statistically significant (Student's t-test) are indicated by [a] ($P < 0.05$); [b] ($P < 0.01$);
PIA = N^6-phenylisopropyladenosine

Table 2

Effect of insulin on the rates of glycogen formation by the stripped soleus muscle preparation of the rat in the presence of adenosine deaminase or adenosine deaminase plus N^6-phenylisoprophyladenosine

Insulin (μunits/ml)	Glycogen formation (μmol . g^{-1} . h^{-1})		
	No addition	Adenosine deaminase	Adenosine deaminase plus PIA
0	1.08 ± 0.16	1.06 ± 0.16	0.79 ± 0.14
1	1.33 ± 0.17	1.01 ± 0.16	0.55 ± 0.10
10	1.30 ± 0.32	1.35 ± 0.14	1.09 ± 0.24
100	3.71 ± 0.48	2.79 ± 0.33	1.84 ± 0.44
1000	5.24 ± 0.77	4.04 ± 0.44	4.80 ± 0.22
10000	6.00 ± 0.44	4.96 ± 0.48	4.61 ± 0.59

Results are presented as means ±SEM for 6 incubations each containing a single muscle preparation from one animal

the analogue plus the deaminase increased the rate of glycolysis above the control. The reason for this effect is not known.

The effects of varying concentrations of insulin on the rate of glycogen synthesis in the presence of adenosine deaminase and adenosine deaminase plus N^6-phenylisopropyladenosine are also shown in table 2. The rate of glycogen synthesis was increased ~4-fold by insulin but this effect was not influenced by the presence of adenosine deaminase or deaminase plus the adenosine analogue. The concentration of insulin that increased glycogen synthesis half-maximally (~100 μunits/ml) was not changed by addition of adenosine deaminase or the deaminase plus the adenosine analogue (not shown).

4. DISCUSSION

The addition of adenosine deaminase to the incubation medium of a stripped soleus muscle preparation increased markedly the sensitivity of glycolysis to insulin; the concentration of insulin required to produce half-maximal stimulation was about 100 μunits/ml in the absence of adenosine deaminase but was about 10 μunits/ml in its presence. This finding suggests that adenosine decreases the sensitivity of muscle glycolysis to insulin; this is supported by the fact that the effect of adenosine deaminase is completely abolished by the presence of an adenosine analogue, N^6-phenylisopropyladenosine, which is not metabolised by the deaminase. It is assumed that the adenosine analogue mimics the normal effect of adenosine. Adenosine per se was not used since it is considered that this compound is continuously produced by muscle to that is would rapidly reach a concentration in the incubation medium that would produce near-maximal effects; a view supported by the effect of addition of the deaminase. Since adenosine is both produced and utilised by tissues and since it appears to affect insulin sensitivity markedly (table 1), the concentration of the hormone that produces half-maximal stimulation of glycolysis should vary according to the concentration of this compound in the incubation medium. This may explain some of the variability reported in the literature in relation to the concentration of the hormone that produces a 50% effect.

It is of considerable interest that the effect of adenosine on muscle is to decrease insulin sensitivity whereas, in complete constrast, it increases insulin sensitivity in adipose tissue. Furthermore in adipose tissue, adenosine deaminase changed the sensitivity of both lipolysis and glucose utilisation whereas in muscle it only affected glycolysis; there was no effect on glycogen synthesis. The reason for this lack of effect on glycogen synthesis is not known but it is speculated that the level of glycogen may play an important role in regulating the rate of glycogen synthesis and its sensitivity to hormones. The lack of effect of adenosine deaminase and the adenosine analogue on glycogen synthesis suggests that effects of adenosine on insulin sensitivity of muscle glycolysis are exerted via a post-receptor mechanism.

The effect of adenosine deaminase on insulin sensitivity in muscle is identical to the effect of exercise-training of the rats [2]; the concentration of insulin required to stimulate glycolysis half-maximally was decreased from ~100–10 μunits/ml by exercise-training and there was no effect on the sensitivity of the progress of glycogen synthesis. It is suggested that the effects of exercise-training on insulin sensitivity are due to changes in the local concentration of adenosine: a decrease in its rate of formation, an increase in its rate of utilisation or a change in the number or sensitivity of adeno-

sine receptors could account for the observed changes in insulin sensitivity. Since exercise-training improves insulin sensitivity in man [10,11] it is tempting to speculate that adenosine is involved. It is well established that insulin-sensitive tissues of animals or patients suffering from obesity or non-insulin dependent diabetes mellitus exhibit decreased sensitivity to insulin. If adenosine is also involved in changes in sensitivity to insulin in these conditions, pharmacological intervention in the processes for production or utilisation of adenosine in skeletal muscle could improve insulin sensitivity and hence improve control of the blood glucose concentration in such patients.

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