Increased insulin sensitivity in soleus muscle from cold-exposed rats: reversal by an adenosine-receptor agonist

Leszek Budohoski+, R.A. John Challiss, Fred J. Lozeman, Bronwyn McManus and Eric A. Newsholme*

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, England

Received 7 August 1984

The effect of 0.5, 2, 7 and 14 days cold exposure at 4°C on insulin sensitivity was investigated in the stripped soleus muscle preparation incubated in vitro. Cold-exposure for 2 or 7 days increased the sensitivity of glycolysis, but did not affect the sensitivity of glycogen synthesis to insulin. Cold-exposure for 0.5 or 14 days had no effect on the sensitivity of either process to insulin. The increased sensitivity to insulin after exposure of animals to the cold for 2 days was completely reversed by addition of the adenosine receptor agonist, 2-chloroadenosine, to the incubation medium. This suggests that cold exposure may increase insulin sensitivity in the muscle, either by a decrease in the concentration of adenosine in the muscle, or by a decrease in the number or affinity of the adenosine receptors.

Cold-exposure *Cold-acclimation* *Insulin sensitivity* *2-Chloroadenosine* *Muscle*

1. INTRODUCTION

Insulin stimulates the rates of glycolysis and glycogen synthesis in muscle [1] and it has recently been shown that adenosine may influence the sensitivity of glycolysis, but not that of glycogen synthesis to insulin [2]. In addition, analogues of adenosine (e.g., 2-chloroadenosine) have been shown to decrease the sensitivity of glycolysis to insulin in the incubated soleus muscle from a rat, while methyl xanthines (e.g., 8-phenyltheophylline) increase insulin sensitivity [3]. Studies on the adenosine receptor suggest that analogues of adenosine act as adenosine agonists for this receptor, whereas the methyl xanthines act as antagonists for this receptor [4,5]. The suggestion that the decreased insulin sensitivity which is characteristic of obesity (insulin resistance) is due, in part, to an increased concentration of adenosine in muscle is supported by the observation that the adenosine antagonist 8-phenyltheophylline completely removes the insulin resistance observed in the incubated soleus muscles from an obese Zucker (fa/fa) rat [6]. It was considered important to test the opposite situation: would an adenosine-receptor agonist restore to normal the increased insulin sensitivity characteristic of a particular physiological condition?

There is some evidence that acute exposure to the cold increases insulin sensitivity in vivo [7,8]. In the present work animals were exposed to the cold (4°C) for various periods of time and the effect of this exposure on insulin sensitivity of glycolysis and glycogen synthesis in the isolated soleus muscle was investigated. At a time when insulin sensitivity was increased, the effect of 2-chloroadenosine on the sensitivity of glycolysis and glycogen synthesis to insulin has been investigated.

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

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V.

2. MATERIALS AND METHODS

Male Wistar rats were obtained from Bantin and Kingman, Hull, and were maintained in the Department's animal house for one week before being transferred to a cold environment (4°C for 0.5, 2, 7 or 14 days). Control rats were maintained at 24°C. Chemicals and enzymes were obtained from sources given in [3,9].

Stripped soleus muscles were prepared and incubated as described in [10] with the modifications described in [9]. Lactate formation was measured spectrophotometrically and radiochemically [9]. The rate of lactate formation was similar for both techniques. Glycogen synthesis was measured by [^{14}C]glucose incorporation into glycogen as described in [11].

Table 1

Effects of different periods of cold exposure on the rates of lactate formation and glycogen synthesis by the isolated stripped soleus muscle in the presence of various concentrations of insulin

Insulin concn. (μU/ml)	Cold exposure (days)	Rates of formation (μmol·g^{-1}· h^{-1})	
		Lactate	Glycogen
1	0	6.57 ± 0.28 (27)	1.43 ± 0.10 (24)
10		6.87 ± 0.29 (27)	1.57 ± 0.11 (24)
100		9.81 ± 0.56 (27)	2.98 ± 0.19 (24)
1000		13.5 ± 0.88 (27)	4.18 ± 0.28 (24)
10000		14.1 ± 0.91 (27)	4.35 ± 0.31 (24)
1	0.5	7.39 ± 0.47 (6)*	1.95 ± 0.17 (6)*
10		8.27 ± 0.49 (6)*	2.20 ± 0.25 (6)*
100		10.7 ± 1.37 (6)	3.58 ± 0.48 (6)
1000		13.7 ± 0.84 (6)	4.84 ± 0.57 (6)
10000		14.0 ± 0.97 (6)	5.26 ± 0.36 (6)
1	2	7.80 ± 1.03 (7)*	1.48 ± 0.23 (9)
10		11.8 ± 1.11 (7)**	1.73 ± 0.16 (9)
100		12.2 ± 0.60 (7)*	2.79 ± 0.37 (9)
1000		15.5 ± 0.93 (7)*	3.51 ± 0.24 (9)
10000		16.0 ± 2.04 (7)	4.13 ± 0.33 (9)
1	7	6.74 ± 0.17 (8)	1.87 ± 0.20 (10)
10		10.1 ± 0.85 (8)**	1.80 ± 0.15 (10)
100		11.9 ± 0.95 (8)*	3.13 ± 0.33 (9)
1000		12.4 ± 0.60 (8)	4.14 ± 0.41 (9)
10000		12.3 ± 0.52 (8)	4.64 ± 0.26 (8)
1	14	6.59 ± 0.70 (5)	1.45 ± 0.25 (5)
10		6.61 ± 0.32 (5)	1.43 ± 0.27 (5)
100		9.86 ± 0.76 (5)	3.44 ± 0.43 (5)
1000		13.3 ± 0.82 (5)	4.83 ± 0.52 (5)
10000		12.4 ± 0.52 (5)	4.53 ± 0.34 (5)

Rates of lactate and glycogen formation were measured as described in section 2 after the indicated periods of cold exposure (4°C). Results are presented as means ± SE with the number of separate incubations given in parentheses. Statistical significance was determined by the Student's t-test, values for different periods of cold exposure were compared to control data obtained in the same experimental protocol. Pooled control data only are given (* $P < 0.05$, ** $P < 0.005$)

3. RESULTS

The effect of cold exposure of rats on the sensitivity of glycolysis (as measured by lactate formation) and glycogen synthesis to insulin in the isolated soleus muscle strip incubated in vitro was investigated. The sensitivity of glycolysis to insulin was increased after 2 and 7 days, but was unaffected by 0.5 and 14 days of exposure to the cold (table 1, fig.1). Thus, after 2 and 7 days of cold exposure there is a statistically significant increase in the rate of glycolysis on raising the concentration of insulin in the incubation medium from 1 to 10 μU/ml insulin; this is not seen in control animals (fig.1). There was no effect of cold exposure (of any duration) on the sensitivity of glycogen synthesis to insulin (table 1).

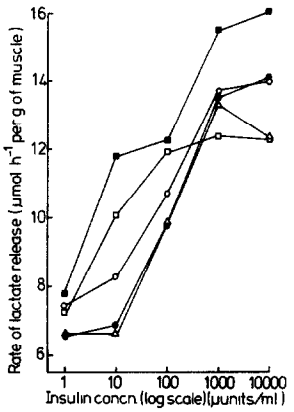

Fig.1. Effect of various concentrations of insulin on the rates of lactate formation by isolated stripped soleus muscles from rats maintained at 24°C (●) or 4°C for 0.5 (○), 2 (■), 7 (□) or 14 (△) days prior to killing.

Table 2

Effects of the adenosine receptor agonist, 2-chloroadenosine, on the rates of lactate and glycogen formation by the incubated soleus muscles isolated from warm-acclimated (24°C) or 2-day cold-exposed (4°C) rats in the presence of various concentrations of insulin

Insulin concn. (μU/ml)	Conditions of incubation	Rates of formation (μmol·g^{-1}· h^{-1})	
		Lactate	Glycogen
1	Muscles from 24°C	6.57 ± 0.28 (27)	1.43 ± 0.10 (24)
10	animals, no additions	6.87 ± 0.29 (27)	1.57 ± 0.11 (24)
100		9.81 ± 0.56 (27)	2.98 ± 0.19 (24)
1000		13.5 ± 0.88 (27)	4.18 ± 0.28 (24)
10000		14.1 ± 0.92 (27)	4.35 ± 0.31 (24)
1	Muscles from 24°C	6.02 ± 1.30 (8)	1.25 ± 0.11 (8)
10	animals, 20 μmol	6.60 ± 0.60 (8)	1.24 ± 0.09 (8)
100	2-chloroadenosine	7.65 ± 0.87 (8)[a]	1.40 ± 0.08 (8)[a]
1000		8.10 ± 0.53 (8)[a]	4.35 ± 0.22 (8)
10000		14.0 ± 1.30 (8)	5.81 ± 0.46 (8)[a]
1	Muscles from 4°C	7.80 ± 1.03 (7)	1.48 ± 0.23 (9)
10	animals, no additions	11.8 ± 1.11 (7)	1.73 ± 0.16 (9)
100		12.2 ± 0.60 (7)	2.79 ± 0.37 (9)
1000		15.5 ± 0.93 (7)	3.51 ± 0.24 (9)
10000		16.0 ± 2.04 (7)	4.13 ± 0.33 (9)
1	Muscles from 4°C	6.58 ± 0.24 (9)[b]	1.52 ± 0.27 (9)
10	animals, 20 μmol	7.32 ± 0.74 (9)[b]	1.66 ± 0.23 (9)
100	2-chloroadenosine	7.16 ± 0.98 (9)[b]	1.92 ± 0.08 (9)[b]
1000		11.4 ± 1.92 (9)[b]	3.24 ± 0.24 (9)
10000		15.9 ± 2.30 (9)	4.30 ± 0.42 (9)

Results are presented as means ± SE with the number of separate incubations given in parentheses. Statistical significance was determined by Student's *t*-test and is indicated by [a] control *vs* control + 2-chloroadenosine, $P < 0.05$; [b] 2-day cold-exposed *vs* 2-day cold-exposed + 2-chloroadenosine, $P < 0.05$

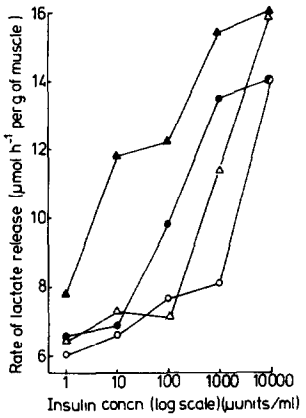

Fig.2. Effect of various concentrations of insulin on the rates of lactate formation by isolated stripped soleus muscles from rats maintained at 24°C (●, ○) or 4°C (▲, △) for 48 h prior to killing. The effect of 20 μM 2-chloroadenosine (○, △) on insulin sensitivity.

The increased sensitivity of glycolysis to insulin after 2 days cold exposure was chosen as the period to investigate the effect of 2-chloroadenosine. The addition of 2-chloroadenosine to the incubation medium decreased the sensitivity of glycolysis to insulin in soleus muscle from control animals (in confirmation of previous work) and completely removed the improvement in sensitivity observed in muscles from the cold-exposed animals (table 2, fig.2). 2-Chloroadenosine decreased slightly the sensitivity of glycogen synthesis to insulin in soleus muscle from both control and cold-exposed animals (table 2).

4. DISCUSSION

There are only a few conditions during which the sensitivity of glucose utilisation to insulin is increased; these include exercise-training [12,14], starvation [15] and, as is shown in this communication, cold-exposure. The effect of cold-exposure has been investigated in the isolated soleus muscle strip of the rat by studying the rates of two processes: lactate formation as an index of the rate of glycolysis and glycogen synthesis by the incorporation of [^{14}C]glucose into glycogen.

When the percentage stimulations of glycolysis and glycogen synthesis are plotted against the insulin concentration, it is possible to calculate approximate values for the concentration of insulin required to stimulate the process by 50%: for glycolysis in soleus muscles from control animals this value is about 100 μU/ml, which is similar to that obtained in previous work [2,12]; similar values were also observed for muscles from animals exposed to the cold for 0.5 and 14 days. However, after 2 and 7 days cold-exposure the concentration of insulin required to stimulate glycolysis by 50% was about 10 μU/ml.

The addition of 2-chloroadenosine to the incubation medium completely removed the effect of 2 days cold-exposure on the sensitivity of glycolysis to insulin (table 2) and the concentration of insulin required to stimulate glycolysis by 50% was almost 1000 μU/ml (fig.2). The fact that 2-chloroadenosine can acutely abolish the improvement in insulin sensitivity suggests that cold-exposure may improve insulin sensitivity by decreasing the local concentration of adenosine or by decreasing the number or affinity of adenosine receptors in soleus muscle.

Further support for the view that changes in adenosine concentration or adenosine receptor properties may be responsible for physiological (or pathological) changes in insulin sensitivity in soleus muscle is provided by the 'symmetry' of the results with adenosine receptor agonists and antagonists and physiologicalchanges in insulin sensitivity. Thus, improved sensitivity, characteristic of cold-exposure, is removed by an adenosine receptor agonist (table 2), whereas the impaired sensitivity characteristic of obesity is removed by an adenosine receptor antagonist [6]. Further work is needed to examine whether other conditions of modified insulin sensitivity will also respond to adenosine receptor analogues.

It has been pointed out elsewhere that insulin increases the rate of glycolysis (i.e., lactate formation) in skeletal muscle by stimulation of the rate of glucose transport across the cell membrane. Since, under the incubation conditions used in the present work, transport is the flux-generating step for glycolysis [12,13], the increased sensitivity of glycolysis to insulin observed in short-term cold-exposure (2 and 7 days) is probably due to increased sensitivity of the glucose transport process in the soleus muscle. The process of glycogen synthesis contains a separate flux-generating step that is catalysed by glycogen synthase [13], the rate of this process will not necessarily be influenced by

changes in the rate of glucose transport into the cell.

ACKNOWLEDGEMENTS

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 a recipient of a Fellowship from the Wellcome Trust.

REFERENCES

[1] Randle, P.J. and Morgan, H.E. (1962) Vitamins Hormones 20, 199–250.
[2] Espinal, J., Challiss, R.A.J. and Newsholme, E.A. (1983) FEBS Lett. 158, 103–106.
[3] Budohoski, L., Challiss, R.A.J., McManus, B. and Newsholme, E.A. (1984) FEBS Lett. 167, 1–4.
[4] Londos, C., Cooper, D.M.F. and Wolff, J. (1980) Proc. Natl. Acad. Sci. USA 77, 2551–2554.
[5] Wolff, J., Londos, C. and Cooper, D.M.F. (1981) Adv. Cyclic Nucleotide Res. 14, 199–214.
[6] Challiss, R.A.J., Budohoski, L., McManus, B. and Newsholme, E.A. (1984) Biochem. J. 221, 915–917.
[7] Smith, O.L.K. and Davidson, S.B. (1982) Am. J. Physiol. 242, R109–R115.
[8] Vallerand, A.L., Lupien, J. and Bukowiecki, L.J. (1983) Am. J. Physiol. 245, E575–E581.
[9] Challiss, R.A.J., Espinal, J. and Newsholme, E.A. (1983) Biosci. Rep. 3, 675–679.
[10] Crettaz, M., Prentki, M., Zaninetti, D. and Jeanrenaud, B. (1980) Biochem. J. 186, 525–534.
[11] Cuendet, G.S., Loten, E.G., Jeanrenaud, B. and Renold, A.E. (1976) J. Clin. Invest. 58, 1078–1088.
[12] Espinal, J., Dohm, G.S. and Newsholme, E.A. (1983) Biochem. J. 212, 453–458.
[13] Newsholme, E.A. and Leech, A.R. (1983) in: Biochemistry for the Medical Sciences, pp.583–586, Wiley, Chichester, New York.
[14] Richter, E.A., Geretto, L.P., Goodman, M.N. and Ruderman, N.B. (1982) J. Clin. Invest. 69, 785–793.
[15] Brady, L.J., Goodman, M.N., Kalish, F.N. and Ruderman, N.B. (1981) Am. J. Physiol. 240, E184–E190.