INSULIN ACTIVATES GLYCOGEN SYNTHASE IN PHOSPHORYLASE KINASE DEFICIENT MICE

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1. Introduction

Glycogen synthase, the rate limiting enzyme in glycogen synthesis, is regulated by a phosphorylation–dephosphorylation mechanism. The enzyme can exist as either a high activity dephosphorylated *a*-form, whose activity is enhanced only slightly by glucose-6-phosphate, or as low activity phosphorylated *b*-forms, which can be activated considerably by glucose-6-phosphate.

An important action of insulin in skeletal muscle is its ability to activate glycogen synthase and thereby stimulate the deposition of glycogen. The effect is observed in diaphragm [1] and soleus muscle [2] in the absence of glucose in the incubation medium, indicating that it is not a consequence of the increased transport of glucose into muscle which is also stimulated by insulin [3,4]. Insulin increases the activity ratio of glycogen synthase (defined as the activity in the absence of glucose-6-phosphate divided by the activity in the presence of glucose-6-phosphate). It therefore produces kinetic changes that are equivalent to a net decrease in the extent of phosphorylation of the enzyme, and such a decrease in the phosphate content of rabbit skeletal muscle glycogen synthase has been demonstrated following the administration of the hormone in vivo [5].

These experiments indicate that insulin either decreases the activity of glycogen synthase kinase or increases the activity of glycogen synthase phosphatase. However, this apparently simple picture is complicated by the realization that glycogen synthase can be phosphorylated at multiple sites by at least 3 different protein kinases, namely cyclic AMP-dependent protein kinase [6,7], phosphorylase kinase [8–11] and an enzyme termed glycogen synthase kinase-3 [9]. These findings raise the question of which glycogen synthase kinase is under the control of insulin.

Phosphorylase kinase phosphorylates glycogen synthase and glycogen phosphorylase at similar rates in vitro [11] suggesting that phosphorylation of glycogen synthase by this enzyme may be physiologically significant. ICR/IAn mice have $< 0.2\%$ of normal phosphorylase kinase activity in their skeletal muscles [12,13] and the absence of calcium-dependent glycogen synthase kinase activity in muscle extracts prepared from ICR/IAn mice was one of the strongest pieces of evidence which demonstrated that this enzyme was identical to phosphorylase kinase [11]. The existence of this mutant strain therefore appeared to allow a direct test of the possibility that insulin increases the activity of glycogen synthase by decreasing the activity of phosphorylase kinase. The results of these experiments are described in this paper.

2. Materials and methods

Mice of the ICR/IAn strain carrying the gene for skeletal muscle phosphorylase kinase deficiency and control strains, C3H/He-mg and Swiss Albino, were obtained from our own colonies. Monocomponent insulin was from Novo Labs. Ltd.

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Mice were killed by cervical dislocation. Each soleus muscle was removed, attached to a stainless steel holder and suspended in 1.5 ml Krebs-Ringer bicarbonate buffer containing 20 mg/ml defatted bovine serum albumin and 2.0 mM sodium pyruvate, in a 20 ml flat-bottomed glass container. The tubes were sealed with rubber stoppers and gassed in O_2:CO_2 (95:5, v/v) during a 15 min preincubation period and for the first 5 min of the incubation. In order to allow for the variation in the basal activity ratio of glycogen synthase from animal to animal, paired soleus muscles from one mouse were used in each experiment. One muscle was incubated in the presence of insulin and the other muscle was used as a control. The final concentration of insulin was 0.2 μg/ml. At the end of the incubations the muscles (6–8 mg) were removed and freeze clamped in liquid nitrogen. The frozen muscle was powdered with a pestle pre-cooled in liquid nitrogen, and sonicated at 0°C for 10–20 s in 0.5 ml 50 mM Tris–HCl (pH 8.2)–5.0 mM EDTA–100 mM KF. The homogenates were centrifuged at 10 000 × *g* for 5 min and the supernatants decanted and assayed immediately [2].

Glycogen synthase was assayed at pH 7.8 as described [14] in the presence of 10 mM Na_2SO_4 and 50 mM KF [2]. The activity ratio of glycogen synthase was defined as the activity in the absence of glucose-6-phosphate divided by the activity in the presence of 7.2 mM glucose-6-phosphate multiplied by 100. Phosphorylase kinase was assayed at pH 8.2 in the presence of calmodulin using purified phosphorylase *b* and one unit of activity was that amount which phosphorylated 1.0 nmol phosphorylase *b*/min at 30°C [15]. Glycogen was measured by the method in [16]. Protein was measured in the muscle supernatants by the method in [17], and in the muscle homogenates (termed total protein) by the method in [18].

3. Results

3.1. *Phosphorylase kinase activity in soleus muscles*

The specific activity of phosphorylase kinase was found to be 0.10 units/mg of supernatant protein in the soleus muscle of ICR/IAn mice, compared to 6.6 units/mg in C3H/He-mg mice. This compared with 0.06 units/mg in mixed skeletal muscle of ICR/IAn mice and 43 units/mg in mixed skeletal muscle from the limbs and back of C3H/He-mg mice. ICR/IAn mice therefore contain only trace residual phosphorylase kinase activity in their soleus muscles as demonstrated in mixed skeletal muscles [12,13].

Table 1
Effect of insulin on glycogen synthase in soleus muscle of phosphorylase kinase deficient mice and control mice

		Phosphorylase kinase-deficient (ICR/IAn)				Control (Swiss albino and C3H/He-mg)			
Incubation	Time	*n*	Glycogen synthase activity ratio	Δ	*P*	*n*	Glycogen synthase activity ratio	Δ	*P*
− Insulin	5	5	29.3 ± 2.0	1.4 ± 1.1	N.S.	7	37.5 ± 2.3	4.7 ± 1.3	< 0.02
+ Insulin			30.7 ± 1.6				42.2 ± 2.2		
− Insulin	10	5	25.2 ± 1.5	6.0 ± 1.4	< 0.02	7	38.5 ± 2.3	6.0 ± 1.3	< 0.01
+ Insulin			31.2 ± 2.4				44.5 ± 2.7		
− Insulin	20	7	24.8 ± 1.8	8.8 ± 1.9	< 0.01	15	39.4 ± 1.8	11.3 ± 0.7	< 0.001
+ Insulin			33.6 ± 2.0				50.7 ± 1.8		
− Insulin	30	7	27.0 ± 2.2	11.9 ± 1.5	< 0.001	9	36.5 ± 1.0	13.3 ± 1.2	< 0.001
+ Insulin			38.9 ± 2.6				49.8 ± 1.2		
− Insulin	50	4	24.4 ± 2.7	14.2 ± 1.7	< 0.01	2	31.1 ± 0.3	14.7 ± 2.6	–
+ Insulin			38.6 ± 3.6				45.8 ± 2.8		

Swiss albino mice were used as controls at 5, 10, 20 (n = 11), and 30 (n = 6) min and C3H/He-mg mice at 20 (n = 4), 30 (n = 3) and 50 min points. The two soleus muscles from one mouse were treated as a pair, one being incubated without and one with insulin. The glycogen synthase activity ratio is the activity of glycogen synthase in the absence of glucose-6-phosphate divided by the activity of glycogen synthase in the presence of 7.2 mM glucose-6-phosphate multiplied by 100. Δ is the increase in the glycogen synthase activity ratio brought about by insulin. Results are expressed as the mean of n muscles ± standard error of the mean. The statistical significance of the effect was assessed by using Student's t-test

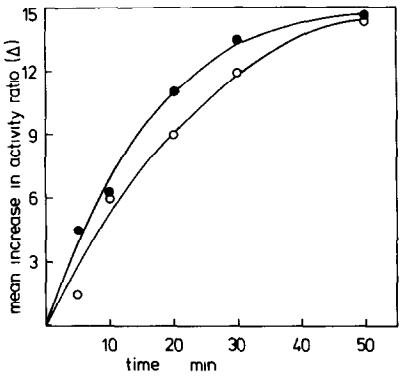

Fig.1. Effect of insulin on the activity ratio of glycogen synthase in ICR/IAn mice (○) and control mice (●). The results show the mean increase in activity ratio plotted as a function of time. The graph is plotted from the data shown in table 1.

3.2. *Effect of insulin on the activity ratio of glycogen synthase in ICR/IAn mice*

Insulin has been shown to increase the activity ratio of glycogen synthase in soleus muscle of Swiss albino mice [2]. The activity ratio increased from 36–48, the time required for half-maximal effect being about 12 min.

The results of identical experiments carried out with phosphorylase kinase-deficient ICR/IAn mice and control mice are shown in table 1 and fig.1. It can be seen that the activity ratio of glycogen synthase in ICR/IAn mice was increased in the presence of insulin in a very similar manner to that observed in the control strains, the time required for half-maximal effect being ~16 min. The increased activity was not altered by dialysing the extracts or treating them with Dowex AG1-X4 resin to remove glucose-6-phosphate. This ruled out the possibility that insulin had activated glycogen synthase by increasing the intracellular concentration of glucose-6-phosphate.

It should also be noted that the basal activity ratio of glycogen synthase was 26 in ICR/IAn mice, which was lower than in control mice (36 in C3H/He-mg and 39 in Swiss albino mice), although the total glycogen synthase activities measured in the presence of glucose-6-phosphate were similar (24 U/mg total protein in ICR/IAn mice and Swiss albino, and 18 U/mg in C3H/He-mg mice). The glycogen content of the soleus muscles of ICR/IAn mice was much higher, being 19.2 μg/mg total protein, compared with 6.9 μg/mg in Swiss albino mice and 4.2 μg/mg in C3H/He-mg mice. This inverse correlation between the activity ratio of glycogen synthase and the glycogen content has been noted using skeletal muscle from the hind limbs [19].

4. Discussion

The results described in this paper show that insulin increases the activity ratio of glycogen synthase in the soleus muscle of ICR/IAn mice, even though this muscle contains only 1.5% of normal phosphorylase kinase activity. The increase in the activity ratio of glycogen synthase in response to insulin is identical to that obtained with control strains, and the time required to achieve a half-maximal effect is similar.

The results therefore exclude the possibility that insulin increases the activity ratio of glycogen synthase by decreasing the activity of phosphorylase kinase. In addition, it should be mentioned that phosphorylase kinase is completely dependent on calcium ions for activity [20] and the phosphorylation of glycogen synthase by phosphorylase kinase is the only known mechanism by which glycogen synthase is regulated by calcium ions. The results therefore also imply that the action of insulin on glycogen synthase activity is unrelated to decreases in the intracellular concentration of calcium ions.

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