Phosphorylation of hexokinase in insulin resistance

ALISON L. SANDERSON, GEORGE K. RADDA and BRENDAN LEIGHTON

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

Regulation of hexokinase (HK) is important in controlling glucose fluxes in skeletal muscle. We have observed that fractional hexokinase activity (HKf) is stimulated by insulin but that stimulation is abnormally low in insulin-resistant muscle [1]. Preliminary evidence suggests that phosphorylation of tyrosine residues is required normally to increase hexokinase activity [2]. In the present study, we have investigated whether regulation by phosphorylation is normal in insulin-resistant muscle or whether an altered mechanism could account for the abnormal insulin-mediated stimulation of HKf.

Soleus muscle strips weighing 20-40 mg were isolated from nine to twelve weeks old lean and obese Zucker rats. Rats had been kept under controlled conditions (12h light/12h dark, 23 ± 1°C) and fed standard laboratory chow and water ad libitum, except for 12-14h immediately prior to experimentation when food was removed. Animals were stunned by a blow to the head and killed by cervical dislocation. Muscle strips, tied to stainless steel clips via their tendons, were each placed in a 25ml Erlenmeyer flask containing 3ml Dulbecco's Modified Eagle's Medium, 5mM HEPES and 10U bovine insulin ml-1. The flasks were gassed continually with O2:CO2 (95%: 5%). After 30 min pre-incubation at 37°C, the muscles were transferred to a second flask containing DMEM and HEPES, as before, and 10 or 10,000µU insulin ml-1. After 30 min incubation, soleus muscles were removed from the clips, and homogenised for 10-15 s in ice-cold buffer (250mM sucrose, 5mM HEPES pH 7.4, 5mM MgCl2, 1mM dithiothreitol, 5% dextan 70) using a Teflon pestle homogeniser. In some experiments the homogenisation buffer also contained 2µM okadaic acid (OKA) or 20µM sodium orthovanadate (VAN). One volume of homogenate was added to one volume of assay buffer to initiate the reaction. The total hexokinase activity (HK) activity in the absence of glucose 6-phosphate) and the fractional hexokinase activity (activity in the presence of glucose 6-phosphate) were measured in each homogenate. For the measurement of HKf the assay medium contained (final concentrations) 75mM triethanolamine, pH 7.4, 7.5mM MgCl2, 1.5mM KCl, 2mM dithiothreitol, 0.8mM EDTA, 2.5mM ATP, 1mM glucose ([U-14C]-glucose ml-1), 10mM phosphocreatine, 7U creatine kinase ml-1. The buffer was identical for the measurement of HKf with the addition of (final concentrations) 3mM NADP*, 30mM G6PDH. Two volumes of 90% ethanol (v/v) were added after three min to stop the reaction. An aliquot of the final sample was pipetted onto DE81 ion-exchange discs. The discs were washed in an excess of water and the amount of phosphorylated product was quantified using a Beckman liquid scintillation counter. One unit of activity is defined by the conversion of 1µmol glucose.min-1 at 30°C.

HKf was similar in muscle from lean and obese rats and was not affected by any treatment (results not shown). VAN increased HKf in insulin-sensitive muscle (Fig 1A; p<0.04), equivalent to the effect of incubation with 10,000 µU insulin/ml [1]. The combined effects of 10,000 µU insulin/ml and VAN were not additive (Fig 1A). OKA had no effect on HKf in homogenates from insulin-sensitive muscle (Fig 1A). Insulin-resistant muscle from obese Zucker rats responded very differently. VAN had no effect on HKf but HKf was increased (p<0.02) with the addition of OKA to the homogenisation buffer (Fig 1B). The effect of OKA mimicked the effect of a maximal stimulating concentration of insulin (Fig 1B)[1]. As with the effects of VAN in insulin-sensitive muscle the combined effect of 10,000µU insulin/ml and OKA were not additive in insulin-resistant muscle (Fig 1B).

Figure 1. The fractional hexokinase activity (HKf) in A insulin-sensitive and B insulin-resistant soleus muscle homogenates after incubation with 10 or 10,000µU insulin/ml and homogenisation with protein phosphatase inhibitors vanadate (VAN) or okadac acid (OKA) * p<0.05 cf. control (same insulin concentration) $ p<0.05 10 or 10,000µU insulin/ml (same extraction conditions)

The presence of VAN should inhibit endogenous protein tyrosine phosphatases although it may also act to increase tyrosine kinase activity. Thus, VAN will increase the level of tyrosine phosphorylation. The results support our previous findings [2] that in insulin-sensitive muscle a mechanism involving the phosphorylation of tyrosine residues may be involved in the insulin-mediated regulation of HK activity. The lack of a response to VAN in insulin-resistant muscle may indicate that in the putative phosphorylation site is absent, that the tyrosine kinase activity is defective or that the VAN is unable to inhibit the protein tyrosine phosphatase(s). OKA is an inhibitor of the serine/threonine protein phosphatases PPI and PP2A. Thus, the results suggest that a serine or threonine phosphorylation was involved in the stimulation of HK activity in insulin-resistant muscle but not in insulin-sensitive muscle. The effect of OKA on HKf from insulin-resistant muscle may reflect an adaptive change whereby insulin-resistant muscle can elevate HKf but not to the same degree as in insulin-sensitive muscle. Alternatively, HK may have multiple sites which are phosphorylated in vivo. The modification to HK that occurred when insulin-resistant muscle was homogenised with OKA may already have been present in HK from insulin-sensitive muscle under basal conditions (i.e. incubation with 10µU insulin/ml). In such a case, a response to OKA of HK from insulin-sensitive muscle would not be expected. This situation would be analogous to the regulation of glycogen synthase activity in vivo via the reversible phosphorylation of at least seven residues. In the case of skeletal muscle HK, the phosphorylation of tyrosine, serine and/or threonine residues could be involved.

We have previously implicated regulation of HK activity in vivo as a key component in the mechanism regulating insulin-mediated glucose metabolism and have observed that the regulation is abnormal in insulin-resistant skeletal muscle [1]. This study provides further evidence that the regulation of HK in insulin-resistant muscle is different from regulation in insulin-sensitive muscle. The altered regulation may be centred on a change in protein phosphorylation of HK, directly, or of proteins which are responsible for the regulation of HK. Direct evidence indicating phosphorylation of HK in insulin-sensitive and -resistant muscle is essential as a greater understanding of the regulation of this enzyme may be important for the treatment of carbohydrate intolerance in humans.

1. Diabetic Medicine (1994) 11 (Suppl 2) S31