Exercise signalling to glucose transport in skeletal muscle

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Contraction-induced glucose uptake in skeletal muscle is mediated by an insulin-independent mechanism that leads to translocation of the GLUT4 glucose transporter to the muscle surface membrane from an intracellular storage site. Although the signalling events that increase glucose transport in response to muscle contraction are not fully elucidated, the aim of the present review is to briefly present the current understanding of the molecular signalling mechanisms involved. Glucose uptake may be regulated by Ca^{2+}-sensitive contraction-related mechanisms, possibly involving Ca^{2+}/calmodulin-dependent protein kinase II and some isoforms of protein kinase C. In addition, glucose transport may be regulated by mechanisms that reflect the metabolic status of the muscle, probably involving the 5′AMP-activated protein kinase. Furthermore, the p38 mitogen-activated protein kinase may be involved in activating the GLUT4 translocated to the surface membrane. Nevertheless, the picture is incomplete, and fibre type differences also seem to be involved.

**Contraction: AMP-activated protein kinase: Ca^{2+}/calmodulin-dependent protein kinase:
Protein kinase C: p38 Mitogen-activated protein kinase**

Glucose uptake in skeletal muscle during exercise involves acceleration of several processes compared with the resting state. These processes include increased supply of glucose obtained via increased capillary perfusion, increased membrane transport capacity obtained via translocation of GLUT4 from a designated intracellular storage compartment to the plasma membrane and transverse tubules (Ploug *et al*. 1998) and possibly via increased intrinsic activity of GLUT4 (for review, see Furtado *et al*. 2003). Finally, the metabolism of the incoming glucose has to be increased. The present review will concentrate on mechanisms involved in increasing muscle membrane glucose transport capacity during exercise or muscle contraction.

A helpful way of considering the regulation of muscle glucose transport may be in terms of two levels of regulation, as proposed by Ihlemann *et al*. (1999*b*). One level is related to the stimulation frequency of the motor nerve and thereby the average intramyocellular Ca^{2+} concentration. This signalling pathway may involve Ca^{2+}/calmodulin-dependent protein kinase (CaMK) and some isoforms of protein kinase C (PKC; Fig. 1). The second level of control

is thought to be related to the actual metabolic status of the muscle cell during contraction, as reflected by energy charge, ion balance, pH and substrate levels, and may implicate the 5′AMP-activated protein kinase (AMPK). Thus, glucose transport during contraction may be regulated by several different mechanisms that are probably partly redundant.

Calcium-related mechanisms

Early evidence for a stimulatory role of intracellular Ca^{2+} in increasing muscle glucose transport comes from studies showing that a pharmacologically-induced increase in myoplasmic Ca^{2+} concentration increases glucose transport in non-contraction muscle (Holloszy & Narahara, 1967; Youn *et al*. 1991). Based on data obtained in electrically-stimulated frog skeletal muscle, Holloszy & Narahara (1965) have proposed that glucose transport during contraction is a function determined only by the stimulation frequency of the muscle. In partial agreement
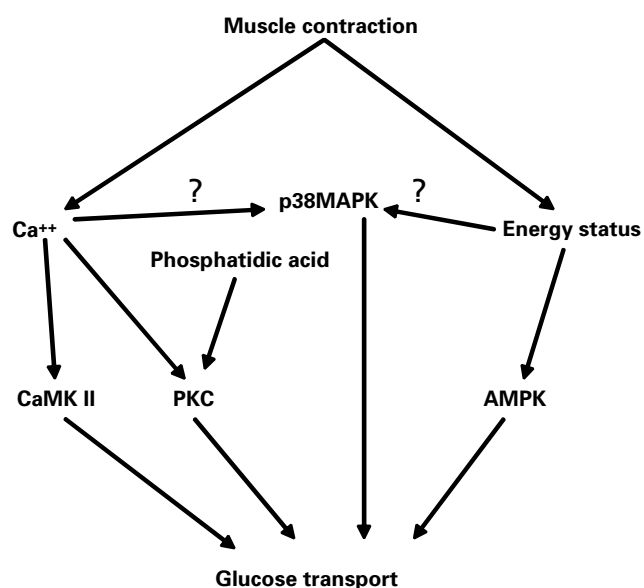

Fig. 1. Schematic presentation of hypothetical activation pathways for glucose transport in contracting skeletal muscle. The calcium-activated pathway may involve calmodulin kinase II (CaMKII), protein kinase C (PKC) isoforms and other still unknown signals, whereas the AMP-activated protein kinase (AMPK) is activated according to the energy status of the muscle. MAPK, mitogen-activated protein kinase.

with these studies, results in incubated rat skeletal muscle have shown that changing the stimulation frequency, while keeping the mechanical output of the muscle constant, to some extent changes glucose transport in parallel with the changes in stimulation frequency (Ihlemann *et al*. 2000). Nevertheless, as glucose transport and stimulation frequency only partially covary, these latter studies also suggest that it is not only the stimulation frequency (and by inference the intracellular Ca^{2+} concentration) that determines the glucose transport capacity of mammalian skeletal muscle.

The signals downstream from Ca^{2+} that are involved in contraction-induced glucose transport have not been identified with certainty, but candidates include CaMKII. An increase in intracellular Ca^{2+} promotes interaction of calmodulin with a diverse group of cellular proteins, including the calmodulin kinases, of which several isoforms exist (Yokokura *et al*. 1997). In fact, calmodulin inhibitors have previously been shown to reduce insulin-stimulated glucose transport in muscle (Youn *et al*. 1994). Experiments with calmodulin inhibitors have revealed inhibition of glucose transport during muscle contraction as well as decreased insulin-induced muscle glucose transport (Ihlemann *et al*. 1999*a*), making it hard to draw firm conclusions about the role of calmodulin during contractions, since the effect of the inhibitors might reflect an effect on muscle glucose transport that is not specific to contraction. On the other hand, a recent report (Wright *et al*. 2004) suggests that the CaMKII inhibitor KN62 decreases contraction-induced glucose transport by about 50 % in incubated rat epitrochlearis muscle, but there is no mention of whether the inhibitor also affects muscle force

production. In addition, it has been reported recently that CaMKII autonomous activity is increased in human skeletal muscle during exercise (Rose & Hargreaves, 2003). Thus, at present there is increasing evidence implicating calmodulin or calmodulin-dependent kinase in contraction-induced glucose transport, but more definitive evidence may be derived from experiments using molecular deletion or overexpression of the enzyme.

Other potential candidates for downstream targets of Ca^{2+} include PKC and, in particular, the Ca^{2+}-sensitive conventional protein kinase PKC isoforms α, β and γ because of the following observations. Several years ago it was shown that total PKC activity to some extent translocates from the cytosol to the particulate fraction (suggesting activation) with muscle contraction (Richter *et al*. 1987). Furthermore, muscle contraction also increases the concentration of diacylglycerol (Cleland *et al*. 1989), which activates the conventional and novel isoforms of PKC. In addition, administration of the PKC inhibitor calphostin C to incubated and perfused muscle has been shown to partially inhibit contraction-induced glucose transport in a fibre-type specific manner (Wojtas-zewski *et al*. 1998; Ihlemann *et al*. 1999*a*). Since calphostin C is thought to block primarily diacylglycerol-sensitive PKC, these findings implicate the conventional and/or novel isoforms in contraction-induced glucose transport, although a blocking effect of calphostin C on atypical PKC (aPKC) also cannot be ruled out (RV Farese, personal communication). Recent evidence has implicated several of the PKC isoforms (and especially the aPKC) in insulin-induced glucose transport in several cell types, including skeletal muscle (Farese, 2002). Furthermore, it has been shown recently that treadmill running in mice (Chen *et al*. 2002) and bicycle exercise in human subjects (Beeson *et al*. 2003; Nielsen *et al*. 2003) increases the activity of aPKC and abundance and phosphorylation of PKCζ in the membrane fraction (Perrini *et al*. 2004) in skeletal muscle. The mechanism for the increase in aPKC activity might be the contraction-associated increase in phosphatidic acid (Cleland *et al*. 1989), which is known to activate aPKC (Farese, 2002). Although such findings *in vivo* do not necessarily indicate that increased activity of aPKC is involved in exercise-induced glucose transport, the findings are compatible with such a role for aPKC in view of their apparent role in insulin-induced glucose transport. Based on this factor and the previously mentioned *in vitro* indications for PKC involvement in glucose transport, further research into the role of PKC activity in contraction-induced muscle glucose transport is warranted.

Mechanisms related to the metabolic status of the muscle

If glucose transport could only be activated by Ca-sensitive mechanisms associated with excitation–contraction coupling, the regulatory and adaptive capacity of the system would be limited. Thus, contraction-induced muscle glucose transport is probably also regulated by feedback signals related to the metabolic status of the muscle. Evidence of feedback regulation of muscle glucose

transport in both fast- and slow-twitch muscle has been provided by Ihlemann *et al.* (1999*b*, 2001), who varied the load on the muscle while stimulating the muscle electrically at a constant frequency. Muscle glucose transport was found to vary closely with the mechanical output of the contracting muscle and thus by the metabolic stress (as evaluated by nucleotide status). Interestingly, glucose transport also correlated well with the activity of AMPK. Since AMPK is regarded as a fuel sensor activated by a decrease in creatine phosphate:creatine and ATP:AMP, 5′AMPK is a good candidate for a kinase that may regulate muscle glucose transport during cellular stress, such as intense muscle contraction (Winder & Hardie, 1999; Fig. 1).

Another level of feedback regulation may be related to conditions in which glycogen (the major carbohydrate source) is scarce. It has been repeatedly shown, both *in vivo* and *in vitro*, that when muscle glycogen is low glucose uptake is increased during contraction (Gollnick *et al.* 1981; Richter & Galbo, 1986; Hespel & Richter, 1990; Wojtaszewski *et al.* 2003). The mechanism for this phenomenon is related to increased glucose transport and GLUT4 translocation when precontraction muscle glycogen is low (Derave *et al.* 1999) and may be coupled to AMPK, since both at rest and during exercise AMPK activity is higher in muscles with a low glycogen content than in those with a high glycogen content (Derave *et al.* 2000; Wojtaszewski *et al.* 2002, 2003).

AMP-activated protein kinase

AMPK can be activated by 5-aminoimidazole-4-carboxamide-riboside (AICAR) in resting muscle and AICAR also results in activation of muscle glucose transport independently of insulin (Merrill *et al.* 1997; Hayashi *et al.* 1998). Since AMPK is also activated in skeletal muscle during contraction or exercise in both rodent (Winder & Hardie, 1996; Vavvas *et al.* 1997; Hayashi *et al.* 1998; Derave *et al.* 2000; Ihlemann *et al.* 2001) and human skeletal muscle (Chen *et al.* 2000; Wojtaszewski *et al.* 2000, 2003; Nielsen *et al.* 2002), it has been suggested that AMPK may be involved in increasing glucose transport during muscle contraction. In human subjects exercising at 70% of their V_{O_2max} and having either high or low muscle glycogen levels muscle $\alpha 2$ AMPK activity and muscle glucose uptake are higher when glycogen levels are low than when they are high (Wojtaszewski *et al.* 2003). Such covariation of mean values may be interpreted as supportive evidence for the notion that AMPK is involved in exercise-induced glucose uptake, but correlative evidence is not necessarily causative. Further correlative evidence between exercise-induced glucose uptake and AMPK activity can be sought in patients with McArdle's disease (glycogen myophosphorylase deficiency). In these patients exercise-induced glucose uptake is exaggerated compared with control subjects working at the same absolute work intensity (Nielsen *et al.* 2002). If AMPK has a regulatory role in glucose uptake it could be hypothesized that AMPK activity would be enhanced during exercise in these patients. It has been found that glucose utilization,

$\alpha 2$ MPK activity and β acetyl-CoA carboxylase-Ser[221] phosphorylation are enhanced in patients with McArdle's disease during 20 min of exercise when compared with the healthy control subjects exercising at the same absolute exercise load. This finding is consistent with a role for AMPK in exercise-induced glucose uptake (Nielsen *et al.* 2002).

There is also some evidence against a role of AMPK in contraction-induced muscle glucose transport. For instance, in slow-twitch rat muscle dissociation between AMPK activity and glucose transport during electrically-induced contractions has been clearly shown (Fig. 2; Derave *et al.* 2000), suggesting that AMPK activity is not closely related to glucose transport. Furthermore, no significant correlation of the individual data points for glucose utilization and AMPK activation was found in the study of the patients with McArdle's disease (Nielsen *et al.* 2002) or in the experiment with high and low muscle glycogen levels (Wojtaszewski *et al.* 2003). Thus, mean values for exercise-induced AMPK activity and glucose utilization covary during exercise in two different human experimental models but the lack of significant correlations of individual values may indicate that there are other factors controlling exercise-induced glucose uptake.

However, these types of experiments, although physiologically important, do not provide definitive evidence of the involvement of AMPK in exercise-induced muscle glucose uptake. Using genetically-manipulated organisms or cell lines may provide more specific information; however a major question relating to these systems is the extent to which they reflect the situation in an exercising individual. Nevertheless, overexpression of a dominant negative form of AMPK (a dead kinase) in mouse muscle has been shown to abolish the AICAR- and hypoxia-induced glucose transport (Mu *et al.* 2001). This finding indicates that hypoxia and AICAR stimulation may use the same signalling pathway through AMPK to increase
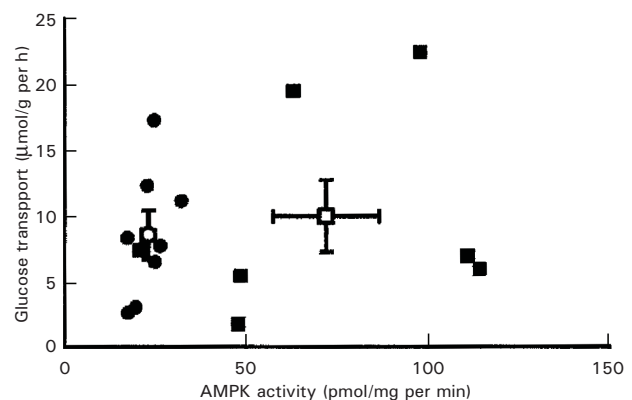

Fig. 2. The relationship between AMP-activated protein kinase (AMPK) activity and glucose transport during contraction in slow-twitch muscles (soleus). Individual data are shown for muscles with high precontraction glycogen levels (●) and muscles with low precontraction muscle glycogen levels (■). (○, □), Corresponding mean values with their standard errors represented by vertical and horizontal bars. (Reproduced, with permission, from Derave *et al.* 2000.)

glucose transport. On the other hand, this study also shows that when muscles are electrically stimulated to contract, glucose transport is only inhibited by approximately 30 % in the kinase-dead muscles compared with the wild-type muscle. This finding may suggest that contraction-induced glucose transport is partly dependent on AMPK. Thus, during contractions *in vitro* some extent of hypoxia, or at least perturbation of the energy status of the muscle, exists that might activate the feedback stimulation of glucose transport via activation of AMPK (Fig. 1). Obviously this pathway will not be activated in the kinase-dead muscles, even though the Ca^{2+}-activated contraction pathway may be unaffected. Although this distinction between the two pathways may be a little tenuous, it probably has some merit in understanding the results obtained in studies involving exercise or contraction. The concept of two different pathways also helps to explain the apparent variation in the role of the different signalling molecules in different exercise conditions. Thus, from this model (Fig. 1) it would be expected that the role of the AMPK-activated feedback pathway would be greatest in severe exercise or contraction in which the energy status of the muscle is compromised, whereas the Ca^{2+}-related feedforward pathway would dominate during less-intense exercise or contraction when AMPK is not activated or only moderately activated (Wojtaszewski *et al*. 2000).

The use of kinase-dead animals (Mu *et al*. 2001) indicates only whether AMPK activity is important for a given biological response but cannot provide any information as to which of the AMPK isoforms might be involved. For this purpose, isoform-specific knock-out of AMPK can be used. AMPK is a heterotrimeric protein consisting of a catalytic α subunit and two regulatory subunits (β and γ). In skeletal muscle the α and β subunits each exist in two isoforms (α_1 and α_2, β_1 and β_2) while the γ subunit exists in three isoforms (γ_1, γ_2 and γ_3; Stapleton *et al*. 1996). The present authors, in collaboration with Vaulont and her colleagues in Paris, have recently carried out studies using $\alpha 1$ AMPK and $\alpha 2$ AMPK knock-out mice. The findings suggest that glucose transport is not affected during *in vitro* electrical stimulation of fast- or slow-twitch skeletal muscle from either $\alpha 1$ or $\alpha 2$ AMPK knock-out mice when compared with muscle from wild-type mice (Jørgensen *et al*. 2004). This finding is particularly interesting in relation to $\alpha 2$ AMPK knock-out mice in which the contraction-induced increase in AMPK-Thr[172] phosphorylation has been shown to be markedly lower than that in the wild-type muscle. Thus, these data suggest either that expression of one AMPK isoform is sufficient to increase glucose transport during contraction, or that AMPK is not necessary for contraction-induced muscle glucose transport.

Another interesting finding derived from studies of AMPK knock-out mice is that AICAR-induced glucose transport and AMPK activation are completely abolished by $\alpha 2$ AMPK knock-out but unaffected by $\alpha 1$ AMPK knock-out (Jørgensen *et al*. 2004). Interestingly, in the muscle from the $\alpha 2$ AMPK knock-out mouse AICAR induces a doubling of $\alpha 1$ AMPK activity while glucose transport is completely unaffected. Thus, in muscle from $\alpha 2$ AMPK knock-out mice, in which a comparable increase in $\alpha 1$ AMPK activity occurs during contractions, it is unlikely that this increase in $\alpha 1$ AMPK activity has an effect on glucose transport during contraction. If this absence of an effect is confirmed, then the normal contraction-induced glucose transport in muscle from $\alpha 2$ AMPK knock-out mice (in which the AMPK activity stems from the $\alpha 1$ isoform) indicates that AMPK is not necessary for a normal increase in muscle glucose transport during contraction.

Mitogen-activated protein kinases

The mitogen-activated protein kinases (MAPK) belong to a superfamily consisting of at least three parallel and distinct MAPK pathways, which include the extracellular-regulated kinases and the two stress-activated protein kinase cascades, c-jun N-terminal kinase and p38 (for review, see Widegren *et al*. 2001). Recently, a potential role of p38 MAPK in contraction and insulin stimulation of glucose transport in skeletal muscle has been suggested (for review, see Furtado *et al*. 2003). Based on studies with p38 MAPK inhibitors and cell lines overexpressing a dominant negative p38 MAPK, it has been suggested that the p38 MAPK may be involved in increasing glucose transport by activating the GLUT4 transporter, although p38 MAPK does not seem to be involved in GLUT4 translocation to the cell surface. As p38 MAPK is activated by muscle contraction (Widegren *et al*. 2001) it might be involved in activating glucose transport during exercise.

Conclusion

Contraction-induced glucose uptake seems to be regulated by Ca-related mechanisms as well as mechanisms related to the metabolic status of the muscle. Elevated intracellular Ca^{2+} levels during contraction activate signalling pathways that may include CaMKII and PKC, whereas AMPK is activated when the metabolic status of the muscle is compromised. The p38 MAPK may also be involved in contraction-induced glucose transport. Evidence from mice in which a dominant negative AMPK is overexpressed suggests that AMPK may be responsible for 30–40 % of contraction-induced muscle glucose transport, whereas recent results indicate that knock-out of either $\alpha 2$ or $\alpha 1$ AMPK does not affect contraction-induced muscle glucose transport. Thus, although there is little doubt that pharmacological activation of AMPK leads to increased glucose transport in resting skeletal muscle, direct evidence for the involvement of AMPK in glucose transport during muscle contraction is limited.

Acknowledgements

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