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Fine-tuning insulin and nitric oxide signalling by turning up the AMPs: new insights into AMP-activated protein kinase signalling

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Abbreviations AMPK: AMP-activated protein kinase · AICAR: 5-amino-4-imidazolecarboxamide ribonucleoside · GSK3: glycogen synthase kinase 3 · mTOR: mammalian target of rapamycin · NO: nitric oxide · PI3-kinase: phosphatidylinositol 3-kinase · S6K: p70 S6 kinase

The regulation of glucose homeostasis is critical for the proper functioning of the body. Unsurprisingly, multiple molecular pathways have evolved to regulate glucose uptake and nutrient metabolism. Under postprandial conditions, when plasma glucose is elevated, insulin stimulates glucose uptake in skeletal muscle, adipose tissue and the heart via activation of phosphatidylinositol 3-kinase (PI3-kinase). The anabolic action of insulin also increases glycogen synthesis via inactivation of glycogen synthase kinase 3 (GSK3), and protein synthesis by stimulation of mammalian target of rapamycin (mTOR).

Under conditions where cells are deprived of nutrients, energy-consuming processes such as protein synthesis are downregulated to conserve energy. The AMP-activated protein kinase (AMPK) is a metabolic sensor that is activated when the energy state of the cell is low, such as during hypoglycaemia, or following muscle contraction, exercise or ischaemia [1]. During these periods of energy substrate deprivation, AMPK activates ATP-producing catabolic reactions, while inactivating ATP-consuming pathways. AMPK activates glucose uptake by stimulating GLUT4 translocation to the cell membrane. Furthermore,

AMPK induces fatty acid oxidation by direct phosphorylation of acetyl-CoA decarboxylase [2]. Conversely, AMPK inactivates mTOR by phosphorylation of tuberous sclerosis tumour suppressors [1], thereby decreasing protein synthesis during times of need.

Insulin is widely recognised as a critical hormone for signal transduction along pathways that promote the storage of nutrients during times of plenty. Conversely, AMPK signalling is activated during times of energy deprivation. Whether insulin- and AMPK-signalling pathways are co-activated under any physiological conditions remains to be elucidated. Pharmacological activators of AMPK, including metformin and thiazolidinediones, are routinely used for the treatment of diabetes. As cross-talk between these two pathways may elicit reciprocal responses, it is critical that the interaction between insulin and AMPK pathways is resolved.

In this issue of *Diabetologia*, Longnus et al. provide evidence that the insulin-stimulated signalling cascade downstream of protein kinase B is potentiated following AMPK activation in the heart in vivo, including enhanced insulin-stimulated p70 S6 kinase (S6K) phosphorylation [3]. This is in contrast to several studies indicating that AMPK activation is associated with reduced S6K phosphorylation [4]. The working hypothesis considers that during energy depletion AMPK is activated, leading to the downregulation of mTOR–S6K signalling such that protein synthesis is decreased and energy expenditure is reduced (Fig. 1). The authors acknowledge that AMPK activation prior to insulin stimulation may explain the observed enhancement. However, 5-amino-4-imidazolecarboxamide ribonucleoside (AICAR), a pharmacological activator of AMPK, does not lead to a subsequent increase in insulin-induced glucose uptake in primary human myocytes [5], indicating tissue-specific differences between cardiac and skeletal muscle cells in response to AMPK activation. Thus, temporal and tissue-dependent factors need to be taken into consideration when evaluating metabolic responses in different tissues.

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Fig. 1 Putative effects of AMPK activation on metabolic adaptations. AMPK inhibits mTOR/S6K activation. However, activation of AMPK prior to insulin stimulation could also potentiate insulin-induced mTOR/p70^{S6K} activation. Furthermore, AMPK activates NO synthase (NOS), which increases NO production, leading to increased glucose uptake. *IRS1* insulin receptor substrate 1, *PI3K* phosphatidylinositol 3-kinase, *PKB* protein kinase B

In addition to insulin and AMPK, amino acids also play a pivotal role in the regulation of S6K activation. In cultured hepatocytes, amino acid-dependent phosphorylation of S6K and S6 is prevented when AMPK is activated by AICAR, fructose or glycerol [6]. Therefore, nutrient supply could potentially add another layer of complexity to the regulation of mTOR–S6K signalling in an in vivo system such as that adopted by Longnus et al.

The role of AMPK in glucose and lipid metabolism is gaining acceptance through functional genomics [7]. Genetic ablation of the AMPK $\gamma 3$ subunit abolishes AICAR-stimulated glucose uptake in skeletal muscle [2]. Conversely, the glycogen content of skeletal muscle is elevated in transgenic mice expressing an activating mutant (R225Q) of AMPK $\gamma 3$ [2]. Thus, AMPK is critical for the regulation of glycogen metabolism in skeletal muscle. Longnus et al. provide evidence that prior activation of AMPK enhances insulin-mediated phosphorylation of the beta isoform of GSK3 in the heart [3]. Therefore, besides a direct effect of AMPK on glucose and lipid metabolism, AMPK may interact with other hormonal signals, such as the insulin-signalling cascade, to accelerate glycogen synthesis. Since GSK3 is also recognised as an insulin-regulated kinase that mediates transcriptional events [8], AMPK could potentially modulate metabolic regulation at the level of transcription. Indeed, several groups have provided evidence that AMPK plays a critical role in the regulation of gene expression in liver and pancreatic beta cells [9].

In addition to insulin- and AMPK-regulated pathways, signals initiated by nitric oxide (NO) have been shown to stimulate glucose uptake in skeletal muscle (Fig. 1). Early evidence of a role for NO in the regulation of glucose transport in skeletal muscle includes the observations that

in vivo nerve stimulation stimulates NO release [10], and that inhibition of NO synthase blunts glucose uptake induced by in vivo electrically stimulated skeletal muscle contraction [11]. Glucose uptake by a wortmannin-insensitive (PI 3-kinase independent) pathway is increased by NO [12]. Furthermore, inhibition of guanylate cyclase, a downstream effector of NO, abolishes NO-stimulated glucose uptake in skeletal muscle, whereas insulin action on glucose transport is preserved [13]. Therefore, the data thus far indicate that insulin- and NO-stimulated glucose uptake in skeletal muscle recruit different signalling pathways.

What could be the upstream activator of NO-induced glucose transport in skeletal muscle? Incubation of cultured myoblasts with the AMPK activator AICAR increases glucose transport, and this effect is abolished by inhibition of NO synthase activity [13]. Furthermore, glucose transport elicited in response to other stimuli thought to be mediated by the AMPK pathway, such as exposure of cells to hyperosmolarity or a mitochondrial uncoupler, is impaired by pharmacological inhibition of NO synthase activity [13]. Since AMPK is activated during exercise, NO is also likely to mediate exercise-induced glucose transport in skeletal muscle. Nonetheless, when skeletal muscle was forced to contract electrically in vitro, inhibition of NO synthase did not affect glucose transport [12]. Thus, the role of NO synthase in exercise-stimulated glucose uptake remains to be characterised.

In this issue of *Diabetologia*, Henstridge et al. [14] provide evidence that infusion of sodium nitroprusside, an NO-donor, stimulates resting leg glucose uptake in subjects with type 2 diabetes. Given that NO-induced glucose uptake is independent of PI3-kinase, the pathway represents an attractive therapeutic target to control plasma glucose levels in patients with type 2 diabetes, in whom insulin-stimulated PI3-kinase activity is reduced [15]. Interestingly, when NO synthase is inhibited during exercise, subjects with type 2 diabetes show a more pronounced decrease in skeletal muscle glucose uptake as compared with healthy subjects [16]. This suggests that there is a greater reliance on the NO pathway for glucose uptake in individuals with type 2 diabetes. Furthermore, several reports have provided evidence that sodium nitroprusside stimulates glucose uptake in different cells, including cardiomyocytes [17] and adipocytes [18], highlighting the potential glucose-lowering effect of NO donors.

Metabolic pathways are integrated in a complex manner. The interaction between AMPK- and insulin-signalling pathways is one example of the tight regulation of whole-body and tissue-specific energy substrate uptake and utilisation. Efforts to delineate novel pathways, such as the AMPK pathway, that may bypass impaired molecular signalling, such as the insulin-signalling pathway, could conceivably lead to new drug targets for the treatment of metabolic disorders (Fig. 1).

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