When Metabolism Rules Perfusion
AMPK-Mediated Endothelial Nitric Oxide Synthase Activation

Eberhard Schulz, Swenja Schuhmacher, Thomas Münzel

Since the first description of the AMP-activated protein kinase (AMPK) more than 20 years ago, numerous studies have established a pivotal role of this enzyme in the regulation of cellular energy balance. Decreased cellular energy supply (indicated by an increased ATP breakdown and accumulation of AMP) leads to an AMPK-mediated adaptation to allow cellular survival. In doing so, AMPK acts as a cellular “fuel gauge,” such that during nutrient deprivation it will inhibit energy-consuming pathways while it promotes energy producing pathways, e.g., fatty acid oxidation or glycolysis. This simple principle is not only operative at the cellular level, because AMPK also governs whole body energy metabolism by the regulation of food intake in the hypothalamus. Because the delivery of nutrients and oxygen to the tissue requires an intact vasculature, it is not surprising that AMPK also affects vascular growth and function. Previous studies revealed that AMPK promotes the production of endothelium-derived NO, acts as a proangiogenic signal, inhibits vascular smooth muscle proliferation, and prevents endothelial cell death. All these processes preserve and protect vascular function, an important precondition to provide tissue and cells with nutrients through an intact vasculature to maintain cellular energy levels. Because NO plays a fundamental role in vascular homeostasis, the interaction between AMPK and endothelial NO synthase (eNOS) has gathered particular interest. Besides gene expression, the activity of eNOS is largely determined by posttranslational modifications such as multisite phosphorylation, subcellular localization, and eNOS–protein interactions. The eNOS phosphorylation sites associated with enzyme activation include Ser1177 and Ser633, whereas the phosphorylation site at Thr495 inhibits eNOS catalytic activity. Site-specific phosphorylation also occurs at Ser114 and Ser615, but the functional consequences remain unclear. Several upstream kinases which modulate eNOS activity have been identified, including Akt, protein kinase (PK)A, PKC, and Ca\textsuperscript{2+}/calmodulin-dependent protein kinase (CaMK)II (Figure). AMPK enhances eNOS activity by direct phosphorylation at Ser1177 and by promoting its association with heat shock protein 90. Initially, Thr495 phosphorylation by AMPK was reported in isolated enzyme preparations but was absent in cultured endothelial cells. Several physiological stimuli are known to initiate AMPK-mediated eNOS activation such as vascular endothelial growth factor, high-density lipoprotein, adiponectin, shear stress, hydrogen peroxide, ghrelin, thrombin, and estrogen. This has important functional implications in the vasculature, because AMPK-dependent NO production is essential for angiogenesis, allows differentiation of endothelial progenitor cells, limits myocardial ischemia/reperfusion injury, and inhibits platelet aggregation. Moreover, it may contribute to the pleiotropic effects of statins, peroxisome proliferator-activated receptor (PPAR)\textgamma agonists and metformin. In addition to direct eNOS activation, AMPK may also increase NO bioavailability indirectly by antioxidative mechanisms that prevent superoxide triggered NO consumption, e.g., by inhibition of NADPH oxidase activity.

In this issue of Circulation Research, Chen et al demonstrate, for the first time, that AMPK can directly phosphorylate eNOS at Ser633, leading to endothelial NO production. It is known that several stimuli leading to Ser1177 phosphorylation (eg, vascular endothelial growth factor, shear stress, and cAMP) also cause phosphorylation at Ser633. However, in contrast to the Ser1177 phosphorylation site, Ser633-mediated eNOS activation is slower and Ca-independent, so that it may serve to maintain NO production after an initial NO burst induced by Ser1177 phosphorylation. AMPK-dependent Ser633 eNOS phosphorylation was also observed in response to hormonal (adiponectin), physical (shear stress), and pharmacological (atorvastatin) stimuli, suggesting that this pathway is operative during diverse physiological and pharmacological signaling events. Experiments with aortic rings from mice lacking the \(\alpha2\)AMPK isoform showed reduced eNOS phosphorylation at Ser1177 and Ser633, implicating \(\alpha2\)AMPK as the major eNOS-regulating isoform. In addition, site-directed eNOS mutations demonstrated that Ser633 eNOS phosphorylation alone is sufficient for NO production, whereas its dephosphorylation prevented AMPK-dependent eNOS activation. Despite these intriguing observations, it remains to be established whether these signaling events are operative in vivo and whether they play a role during physiological or pathological vascular regulation. The variety of stimuli known to activate AMPK would suggest that the enzyme is constitutively active in the vasculature. During vascular disease, increased formation of reactive oxygen species or reactive nitrogen species is a common feature. In this respect, AMPK activation by hydrogen peroxide and peroxynitrite occurs in endothelial...
NADPH, nicotinamide adenine dinucleotide phosphate; O$_2$eNOS phosphorylation is specifically linked to the arterial hypertension. Also, it remains unclear whether these mice are more susceptible to endothelial dysfunction or the development of interesting to know whether these mice are more susceptible to endothelial dysfunction or the development of

Because endothelial cells express predominantly the α2AMPK isoform or just a consequence of changes in total AMPK activity. Because endothelial cells express predominantly the α1-containing AMPK, studies using α1AMPK-deficient mice or cells will clarify this issue. Beneficial effects of increased NO production and bioavailability will become apparent only if they are sustained over time. In this respect, we do not know yet if AMPK-mediated eNOS activation is just a rapid, short-lived response to direct blood flow according to demand or might prevail during a longer period of time. Because NO itself can activate AMPK,7 AMPK-mediated eNOS activation might lead to a sustained NO release owing to a positive feedback loop. Most importantly, the functional relevance of the current findings needs to be confirmed in animal studies with genetic deletion of vascular AMPK isoforms. These investigations might lead to new insights into how changes in cell and tissue metabolism can direct blood flow according to demand. For example, AMPK-mediated eNOS activation might play an important role in the adaptation processes during physical activity, such that tissues with the highest nutrient demand (indicated by activation of AMPK) will receive the highest blood flow owing to AMPK-eNOS–mediated vasodilation. The suggestion that AMPK directs several beneficial processes that are associated with regular physical activity is supported by a recent work from Narkar et al.,8 showing that AMPK activation mimics the effects of exercise training. During vascular disease, such as coronary artery disease with severe stenosis, metabolic stress-dependent vasorelaxation might be even more important, because it allows blood flow in the poststenotic vessel segment and AMPK activation might play an essential role to maintain perfusion and myocardial vitality under these circumstances. This mechanism is also termed metabolic vasomotion and was previously attributed to adenosine formation as an indicator of ATP breakdown. Given our knowledge about the central role of AMPK in the adaptation to metabolic stress and the well-established AMPK-eNOS signaling pathway, it is tempting to speculate that AMPK-mediated eNOS activation is the real link between metabolism and perfusion, thus regulating metabolic vasomotion. Despite this appealing hypothesis, it remains to be established whether energy-dependent mechanisms are the primary means of AMPK regulation in the endothelium.

Several AMPK-mediated signaling pathways governing glucose metabolism can improve glycemic control and have implicated AMPK as a putative molecular target in the treatment of diabetes mellitus. Because anti-diabetic drugs such as PPARγ agonists (glitazones) and metformin have AMPK activating properties and can also improve endothelial function, it is attractive to speculate that the observed protective vascular effects are caused by AMPK-mediated eNOS activation. Because vascular complications are the leading cause of death in diabetic patients, a therapy with AMPK-activating drugs would fulfill 2 im-
important goals: it improves glycemic control and may preserve endothelial function at the same time. However, PPARγ agonists and metformin activate AMPK indirectly by inhibition of the mitochondrial respiratory chain, so that the full potential of pharmacological AMPK activation might be achieved only with the development of more specific AMPK activators without these undesired side effects. Future animal and human studies are warranted and will determine whether AMPK can be established as a novel molecular target in the treatment of vascular disease.

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Disclosures

None.

References


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