AMPK
A regulator of ion channels

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Abbreviations: AICAR, 5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide; AMPK, AMP-activated protein kinase; Ca-AMPK, constitutively active AMPK mutant; CFTR, cystic fibrosis transmembrane conductance regulator; ENaC, epithelial sodium channel; PKA, Protein Kinase A

Ion transport processes are highly energy consuming. It is therefore critical to couple ion transport processes to the metabolic state of the cell. An important player in this coupling appears to be the AMP-activated protein kinase (AMPK). This kinase becomes activated during conditions of cellular metabolic stress and is well-known for its role in promoting ATP-generating catabolic pathways while turning off ATP-utilizing anabolic pathways. Over the past decade AMPK has also emerged as a key regulator of ion channel activity as an increasing number of ion channels are reported to be either directly or indirectly regulated by the kinase. AMPK therefore provides a necessary link between cellular energy levels and ion channel activity.

The AMP-Activated Protein Kinase

The first catalytic subunit of mammalian AMP-activated protein kinase (AMPK) was cloned in 1994. It is a ubiquitously expressed Ser/Thr kinase, which exists as a heterotrimer composed of a catalytic α-subunit and regulatory β- and γ-subunits.

AMPK phosphorylates target proteins containing a \( \Phi(X, \beta)XXS/TXXX\Phi \) (Φ, hydrophobic; β, basic) consensus motif. It is activated in response to an elevated AMP:ATP ratio and phosphorylation of the α-subunit at S172 by upstream kinases such as LKB1 and Calmodulin-dependent protein kinase kinase-β. Binding of AMP to the γ-subunit allosterically activates the kinase. In addition, AMP-binding promotes phosphorylation of S172 by upstream kinases and also prevents dephosphorylation of S172 by protein phosphatases. This complex regulation allows AMPK to detect relatively small changes in the AMP:ATP ratio, making AMPK an excellent regulator of cellular energy homeostasis. Indeed, the first described AMPK substrates were pivotal metabolic enzymes, where AMPK was found to shut off ATP-utilizing anabolic pathways and turn on ATP-generating catabolic pathways.

In addition to its effects on cellular metabolism, AMPK influences a great deal of other cellular processes including cell growth and division, apoptosis, gene transcription, protein synthesis and cell polarization. This review will focus on one of the more recently recognized functions of AMPK, which is its impact on ion channel activity. An overview of the AMPK regulated ion channels reported to date is provided in Table 1.

AMPK Inhibits Ion Channel Activity by Direct Channel Phosphorylation

The concept of AMPK as a regulator of ion channel activity emerged in 2000 with the discovery by Hallows and coworkers that the kinase inhibits the cystic fibrosis transmembrane conductance regulator (CFTR), the ATP-gated chloride channel mutated in cystic fibrosis. By a yeast two-hybrid screen, they identified the AMPK α1 subunit as an interaction partner of CFTR. Functional studies in Xenopus oocytes demonstrated that AMPK can inhibit channel activity by reducing the open probability of the channel. AMPK directly phosphorylates CFTR in vitro and two subsequent studies have identified S768, a previously described inhibitory PKA site, as the primary site of AMPK phosphorylation. Phosphorylation at S768 inhibits PKA stimulation of CFTR gating thereby allowing AMPK to influence the level of CFTR activation caused by the PKA pathway.

Subsequent studies demonstrated that CFTR is not the only ion channel, which is inhibited by AMPK by direct channel phosphorylation. In type I cells of the carotid body, hypoxia is known to cause K\textsuperscript{+} channel inhibition leading to membrane depolarization, calcium entry and subsequent neurosecretion ultimately resulting in changes in respiration. In 2007, Wyatt and coworkers reported that the observed K\textsuperscript{+} channel inhibition was mediated by AMPK. They furthermore found that the BK\textsubscript{Ca} channel, which is partly responsible for the O2-sensitive K\textsuperscript{+} current of type I cells, was inhibited by AMPK activation in HEK293 cells. Though no direct interaction between the kinase and BK\textsubscript{Ca} could be detected in HEK293 cells, the inhibition appears to be a result of direct channel phosphorylation, as AMPK was able to phosphorylate the...
AMPK Inhibits Ion Channel Activity by Nedd4-2 Activation

In 2005, AMPK-mediated inhibition of the epithelial sodium channel ENaC was reported in Xenopus oocytes and polarized mouse collecting duct mpkCCDC14 cells. Interestingly, ENaC did not appear to be a direct target of AMPK as the channel was not phosphorylated by AMPK in vitro and no direct interaction between the kinase and ENaC could be detected. Of note, a Liddle’s syndrome ENaC β-subunit mutant did not respond to AMPK activation. This mutant is characterized by its inability to interact with the E3 ubiquitin ligase Nedd4-2, which normally ubiquitinylates ENaC marking it for endocytosis and degradation. As this mutant is insensitive to Nedd4-2 regulated endocytosis, it suggested that AMPK inhibits ENaC through Nedd4-2. Indeed, this was confirmed in a subsequent study, which reported that Nedd4-2 is a direct target of AMPK and that AMPK activation increases the interaction between the ENaC β-subunit and Nedd4-2. The exact mechanism behind the observed AMPK-induced increase in Nedd4-2 binding to ENaC is currently unknown as are the AMPK phosphorylation sites in Nedd4-2. That ENaC is physiologically regulated by such an AMPK-Nedd4-2 pathway was confirmed by Almaca and coworkers.

More recent studies have demonstrated that the described AMPK-Nedd4-2 pathway is not unique to ENaC. The two potassium channels Kir2.1 and Kv7.1 can also be regulated by this pathway. We demonstrated that during the polarization channel in vitro. Interestingly, a follow-up study demonstrated that AMPK regulates BKca in a splice variant specific manner, which could allow for cell-type specific responses of BKca to AMPK. In accordance with the proposed role of AMPK-mediated BKca channel inhibition in the carotid body, the AMPK-sensitive BKca ‘ZERO’ variant is reportedly expressed in type I cells whereas the non-responsive ‘STREX’ variant is not.

In the report by Wyatt and coworkers, O2-sensitive leak K+ currents of type I cells were also found to be inhibited by AMPK activation. The molecular identity of the currents is still under debate, but most likely is of a TASK-like type. In agreement with an AMPK-regulation of these channels, Dallas et al. reported AMPK-mediated inhibition of TASK-3 channels when expressed in HEK293 cells. This observation is in contrast to data from Kreneisz and coworkers, who found that TASK-1 and TASK-3 channels as well as TASK-1/TASK-3 heteromers did not respond to the AMPK activator 5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide (AICAR). Instead, they found that AMPK activation inhibited the activity of the two related channels TREK-1 and TREK-2. The authors therefore suggest that TREK channels represent the AMPK-inhibited background K+ channels of type I cells. The reason for the different observations on TASK-3 remains unresolved, but might reside in different experimental conditions, in particular the exposure time to AICAR.

Finally, the AMPK γ1 subunit has been demonstrated to bind to the potassium channel KCa3.1 and AMPK inhibits KCa3.1 currents in lung epithelial tissues. The mechanism behind the inhibition has not been determined.

### Table 1. Overview of ion channels affected by AMPK activation.

| Ion channel | AMPK effect | Mode of action | References |
|-------------|-------------|----------------|------------|
| BKca        | Reduction in current amplitude | Direct channel phosphorylation | 23         |
| CFTR        | Decreased open probability | Direct channel phosphorylation (S768) | 20,19,21   |
| ENaC        | Reduction in cell surface expression | Activation of Nedd4–2-mediated endocytosis | 29,28      |
| Kir2.1      | Reduction in current amplitude | Direct channel phosphorylation (S385) | 45,44      |
| Kir6.2      | Reduction in current amplitude | Activation of Nedd4–2-mediated endocytosis | 33         |
| Kv2.1       | Reduction in cell surface expression | Activation of Nedd4–2-mediated endocytosis | 32,31,34   |
| TASK-3      | Reduction in current amplitude | Involves the phosphorylation sites S300 and S333 | 26         |
| TREK-1      | Reduction in current amplitude | Involves the phosphorylation sites S326 and S359 | 26         |
| TREK-2      | Reduction in current amplitude | ? | 25 |
| Kir6.2      | Increased open probability | ? | 40         |
| Kir6.2      | Increased surface expression | ? | 43,42      |
| Kv2.1       | Hyperpolarizing shifts in the current-voltage relationship for channel activation and inactivation | Direct channel phosphorylation (S440) | 38         |
| Nav1.5      | Slowing of open-state inactivation and a hyperpolarizing shift in the voltage-activation curve | ? | 37         |

Shown is a list of the ion channels reported to date to respond to AMPK activation. It is summarized how AMPK activation affects the individual ion channels and what the molecular background for the observed regulation is.
process of MDCK cells, AMPK activation leads to Nedd4-2-dependent endocytosis of surface-expressed Kv7.1 channels followed by lysosomal degradation. Similar results of AMPK activation was reported in polarized mpkCCD cells and collecting duct principal cells from rat kidney slices. In addition, endogenous AMPK activation in Xenopus oocytes co-expressing Kv7.1 and Nedd4-2 downregulated Kv7.1 currents in a Nedd4-2 dependent manner. Altogether the data suggest that Kv7.1 is regulated by a pathway similar to ENaC. Similarly, an AMPK-Nedd4-2 pathway was also reported to inhibit the potassium channel Kir2.1 when exogenously expressed in Xenopus oocytes.

Intriguingly, an increasing number of ion channels are reported to be regulated by Nedd4-2 including Nav1.5, Kv1.3, Kv1.5 and Kv7.2/3 raising the possibility that these channels are also sensitive to AMPK activation through Nedd4-2. AMPK-mediated Nedd4-2 activation could thereby be speculated to be a general cellular mechanism to remove ion channels from the membrane during cellular stress.

**AMPK Can Increase Ion Channel Activity**

The first ion channel reported to display AMPK-facilitated activation was the cardiac sodium channel Nav1.5. Prompted by the observation that mutations in the AMPK γ2 subunit are associated with potentially fatal cardiac arrhythmias, Light and coworkers examined the effects of overexpressing a constitutively active AMPK mutant (CA-AMPK) in rat ventricular myocytes and observed a prolongation of the cardiac action potential. Patch clamp measurements on Nav1.5-expressing Tsa201 cells revealed that the CA-AMPK mutant caused a slowing of channel inactivation and a hyperpolarizing shift of the voltage activation curve, which could provide the explanation for the CA-AMPK-induced action potential prolongation. They therefore suggest that Nav1.5 is a target of AMPK and could contribute to arrhythmias observed in patients with AMPK γ2 mutations.

A recent study added the Kv2.1 potassium channel to the list of AMPK targets. This potassium channel provides the major component of the delayed rectifier Kv current in cortical and hippocampal pyramidal neurons, thereby having a major impact on the firing of action potentials. Ikematsu and coworkers demonstrated that AMPK activation in HEK293 cells resulted in hyperpolarizing shifts in the voltage dependence of Kv2.1 gating. By combining in vitro phosphorylation, mass spectrometry and the use of phosphospecific antibodies, direct phosphorylation of two serine residues (S440 and S537) in the Kv2.1 C-terminus was demonstrated with S440 being the primary site responsible for the observed AMPK effect. In accordance with an AMPK-induced activation of Kv2.1, introduction of active AMPK into cultured hippocampal neurons caused a decrease in the frequency of evoked action potentials. As action potential firing can account for 25–50% of neuronal ATP-turnover, the authors suggest that AMPK regulation of Kv2.1 could serve a protective role by reducing neuronal excitability during conditions of metabolic stress.

**AMPK Regulation of K_{ATP} Currents in Cardiomyocytes and Pancreatic Beta Cells**

Very recently, AMPK was reported to be part of the macromolecular K_{ATP} channel complex of rat cardiomyocytes and AMPK activation was shown to increase the K_{ATP} current in these cells. In inside-out patches from the cardiomyocytes, ZMP (the intracellular metabolite of AICAR) caused strong activation of K_{ATP}. Furthermore, recombinant AMPK activated Kir6.2/SUR2A, the molecular component of the cardiac K_{ATP} current, in transiently transfected COS7L cells demonstrating that the kinase can promote K_{ATP} opening. In agreement with a stimulatory role of AMPK on K_{ATP} channels in heart, the kinase has also been reported to promote K_{ATP} surface-expression in cardiomyocytes. In hypoxia-induced preconditioning of the heart, which protects against myocardial infarction, activation and recruitment of sarcolemmal K_{ATP} channels is involved. Using transgenic mice overexpressing a dominant-negative form of the AMPK α2 subunit, Sukhodub and coworkers demonstrated that the activation and increased surface-expression of K_{ATP} channels observed after preconditioning requires AMPK activity. The mechanism behind the increased surface-expression was, however, not determined.

In pancreatic β cells, the picture is more clouded as AMPK activation has been reported to both promote and inhibit K_{ATP} channel activity. Like reported in cardiomyocytes, AMPK activation also appears to promote K_{ATP} surface-expression in rat pancreatic β cells. However, two other reports suggest an inhibitory role of AMPK. Wang and coworkers observed that application of AICAR to mouse islets in 5–10 mM glucose inhibited K_{ATP} activity and stimulated insulin secretion. In agreement, Chang and coworkers reported that Rosiglitazone, an anti-diabetic drug, caused an AMPK-dependent inhibition of K_{ATP} channels in rat islets. They additionally identified S385 in the Kir6.2 subunit, a previously suggested ERK2 phosphorylation site, as a substrate phosphorylation site for AMPK. The reason for the discrepancy on AMPK mediated effects on K_{ATP} channels in pancreatic β cells has not been solved, but most likely involves differences in the experimental set-ups or possibly non-AMPK related effects of the drugs used. In any case, the contradictory results suggest that regulation of K_{ATP} activity in pancreatic β cells is complex.

**Future Perspectives**

Over the past 12 y AMPK has emerged as an important regulator of ion channel activity. A general aspect appears to be its function to downregulate ion channel activity to preserve energy and prevent dissipation of ionic gradients when transporter and exchanger functions might be compromised during conditions of metabolic stress. In addition, more recent research has expanded the role of AMPK-mediated ion channel regulation to transmission of oxygen-sensing in carotid body cells and stimulation of potassium channel activity to reduce neuronal excitability in energy-lacking conditions. Overall, all of these functions are in line with the role of AMPK as a metabolic sensor that tries
to maintain energy homeostasis. There is no doubt that future studies will reveal even more ion channels as targets of AMPK regulation. Together with investigations of the molecular mechanisms involved in ion channel regulation by AMPK, this will provide important insight into the coupling between AMPK and ion channel activity as well as the physiological aspects of this regulation.

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