Role of the energy sensor AMP-activated protein kinase in renal physiology and disease

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AMP-activated protein kinase (AMPK) is a ubiquitously expressed heterotrimeric kinase that acts as an ultrasensitive cellular energy sensor (119). During energy stress as the level of ATP begins to fall, there is a marked increase in the cellular concentrations of AMP (45). This increase in AMP leads to activation of AMPK via multiple mechanisms (43). Once activated, AMPK acts to restore energy homeostasis by phosphorylating multiple substrates that act both to stimulate energy production and minimize energy consumption. Over the past decade, the biology and biochemistry of the AMPK pathway have been intensively studied in various organs such as the liver, skeletal muscle, and heart. Although AMPK is abundantly expressed in the kidney (29, 118), an understanding of its role in renal physiology and disease is less developed than in other organs. In recent years, however, interest regarding AMPK in the kidney has intensified, with studies describing roles for AMPK in multiple aspects of renal physiology and disease including ion transport (16), podocyte function (113), renal hypertrophy (69), ischemia (95), inflammation (101), diabetes (15, 36, 69), and polycystic kidney disease (122). AMPK is also potentially an important regulator energy metabolism in the kidney. This review gives an overview of AMPK biology and summarizes current knowledge of the functions and regulation of AMPK in the kidney.

Structure and Biology of AMPK

AMPK is a heterotrimer consisting of a catalytic α-subunit and regulatory β- and γ-subunits (98, 118). Each of these subunits exists as multiple isoforms (α1, α2, β1, β2, γ1, γ2, γ3), thereby giving rise to 12 possible heterotrimer combinations (118), with splice variants adding further to the possible diversity (68, 82) (Fig. 1). That the genes encoding the α-, β-, and γ-subunits are each highly conserved in all eukaryotic species (123) is indicative of the fundamental role AMPK plays in cell biology. There do not appear to be major differences in the substrate specificities between the α1- and α2-
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Fig. 1. Structural features of AMP-activated protein kinase (AMPK) subunit isoforms. Known phosphorylation sites are shown. The midsection of the β-subunit contains a carbohydrate binding module (CBM). Regulation of AMPK by AMP is explained by the binding of AMP to the Bateman domains of the γ-subunit. Each Bateman domain consists of 2 cystathione-β-synthase (CBS) domains. Various phosphorylated residues in the α- and β-subunits are shown, includingThr-172 site, which must be phosphorylated by upstream kinases for AMPK to have catalytic activity.

catalytic subunits, although in some cell types the α2-catalytic subunit has a preferential nuclear localization (107). The β-subunits (β1, β2) have conserved central and C-terminal domains and a more variable N terminus. The C terminus of the β-subunit is a subunit-binding domain that is essential for the formation of the αβγ AMPK heterotrimer (52) (Fig. 1). In the middle region of the β-subunit is a carbohydrate-binding module (CBM) that binds glycogen and contributes to the regulation of AMPK activity (49, 103). The activation of AMPK by AMP is explained by its binding to the γ-subunit (1). The C termini of the γ-subunits consist of four tandem cystathione-β-synthase (CBS) domains (111). Two CBS domains combine to form a nucleotide-binding structure termed a Bateman domain (111) (Fig. 1). Structural analysis has demonstrated that each γ-subunit actually has 3 AMP-binding sites (135). Two of the sites can reversibly bind either AMP or Mg\textsuperscript{2+}-ATP and account for the regulation of the kinase, whereas the third site binds AMP irreversibly (135). The major difference between the three γ-subunit isoforms (γ1, γ2, γ3) is the length of their N-terminal extensions, the functions of which remain poorly defined (123).

The activity of AMPK is exquisitely sensitive to cellular energy stress, which is detected as a rising concentration of AMP and an increase in the AMP:ATP ratio. As AMP levels rise, resulting in AMP binding to the Bateman domain of the regulatory γ-subunit (111), AMPK activity is increased by three mechanisms (56). First, there is a direct allosteric effect. Second, AMP binding allows the catalytic loop of the α-subunit to be phosphorylated at residue Thr172 by one of at least three potential upstream AMPK kinases (131, 132, 136). Phosphorylation at Thr172 is essential for activation of AMPK and increases its activity by ~100-fold (47). Moreover, there is a ~1,000-fold activation of AMPK by the combined effects of upstream kinases and saturating concentrations of AMP (121). The third mechanism is that AMP binding inhibits dephosphorylation of Thr172 by protein phosphatases, such as protein phosphatase 2C-α (109). In fact, recent evidence suggests that inhibition of dephosphorylation by phosphatases may be the major mechanism by which AMP binding causes increased phosphorylation of Thr172 (109, 121). These three effects of AMP make the system very sensitive to small increases in AMP concentration. All three effects are also antagonized by high concentrations of ATP. Because all eukaryotic cells express very active adenylate kinase, which maintains the reaction (2ADP ↔ AMP + ATP) close to equilibrium at all times, the cellular AMP:ATP ratio varies approximately as the square of the ADP:ATP ratio (45), making it a very sensitive indicator of cellular energy status. Of note, the half-maximal concentration of AMP required to activate AMPK is <2 μM (121), consistent with the idea that AMPK has tonic cellular activity in the absence of metabolic stress. Indeed, our recent data suggest that tonic phosphorylation of CFTR by AMPK in bronchial epithelial cells plays an important role in preventing channel activation in the absence of PKA stimulation (59).

The upstream AMPK kinase LKB1 was first identified as a tumor suppressor protein mutated in patients with Peutz-Jeghers syndrome, which is characterized by the development of benign hamartomatous polyps in the colon (53). LKB1 exists as a complex with two accessory subunits, termed STRAD and MO25 (46, 132). The LKB1 complex itself appears not to be activated by AMP, with the effect of the nucleotide making AMPK a better substrate for LKB1, while at the same time making it a worse substrate for protein phosphatases that dephosphorylate Thr172 (109). The calcium/calmodulin-dependent kinase kinases (CaMKKs) are also capable of activating AMPK by phosphorylation of Thr172 (50, 131). The in vivo evidence for regulation of AMPK by CaMKKs is strongest for the β isoform (CaMKKβ) (50). Regulation of AMPK by CaMKKβ appears to be independent of the AMP:ATP ratio and is primarily regulated in response to changes in intracellular calcium concentration (50, 131). The tissue distribution of CaMKKβ appears more restricted than LKB1. CaMKKβ is expressed primarily in the brain but is also expressed in testis, thymus, and T cells (4). There is also evidence that in some cell types transforming growth factor (TGF)-β-activated kinase-1 (TAK1) can function as an upstream AMPK kinase (136).

In addition to being regulated at a single-cell level by the level of energy stress, AMPK can also be regulated by extracellular signals such as hormones and cytokines. For example, AMPK is activated in endothelial cells by ligands such as thrombin (116) and bradykinin (96) via a pathway that signals through the Ca\textsuperscript{2+}/CaMKKβ pathway. Of particular interest is the regulation of AMPK by adipokines such as adiponectin (138) and leptin (93). The effect of these hormones on AMPK
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in diverse sites such as skeletal muscle and the hypothalamus is important in the regulation of whole body energy homeostasis (56). For example, inhibition of AMPK by leptin in the hypothalamus leads to a reduction in food intake (92), whereas activation of AMPK by leptin in skeletal muscle causes an increase in glucose uptake and fatty acid oxidation (93).

The best characterized pathways and substrates that are regulated by AMPK are those involved in energy metabolism. Detailed reviews of the substrates and pathways regulated by AMPK have been published elsewhere (44, 56, 58, 123). In general, AMPK acts to restore energy balance by stimulating pathways that lead to ATP synthesis and inhibiting pathways that lead to ATP consumption. For example, AMPK phosphorylates and inhibits both isoforms of acetyl-CoA carboxylase (ACC1 and ACC2), which is the rate-limiting enzyme in fatty acid synthesis (26). Phosphorylation of ACC by AMPK also stimulates fatty acid oxidation by increasing mitochondrial fatty acid uptake (26). AMPK also negatively regulates 3-hydroxy-3-methylglutaryl-CoA reductase, which is the rate-limiting enzyme for cholesterol synthesis (21). Among the other diverse biological processes shown to be regulated by AMPK are cellular nutrient (glucose and fatty acid) uptake (51, 88), protein synthesis (12, 25, 48, 65), gene transcription (139), inflammation (101), ion transport (38), autophagy (76), cellular polarity (11), and nitric oxide synthesis (19).

Expression of AMPK in the Kidney

The pattern of AMPK subunit expression in the mammalian kidney has been described in the rat (15, 29). In the kidney, the α1-subunit is the predominant catalytic isoform, although the α2-subunit is also detectable (29). In the rat kidney, β2 is predominant (15, 29), although we have found β1 to be predominant in the mouse kidney (Power DA, unpublished observations). Both the γ1- and γ2-subunits are expressed at similar levels in the kidney, with the γ2-subunit appearing to exist as the short form (29). As would be expected, the muscle-specific γ3-isofrom has never been detected in kidney tissue. While AMPK α1 is ubiquitously expressed throughout the kidney, immunostaining with an antibody specific for activated AMPK that is phosphorylated at θThr172 shows strongest staining at the apical surface of cortical thick ascending limbs and the macula densa (29, 95). Staining for θThr172 AMPK has also been detected on the basolateral surface of collecting ducts (29). A limitation of current knowledge of AMPK expression and its activity in the kidney is a lack of data regarding differences in expression between different cell populations within the kidney. This problem relates to the fact that most studies of AMPK expression have focused on expression in whole lysates or single cell types. Further characterizations with immunolocalization studies are required to determine expression differences that might exist in different cell populations, which could be important because there are known marked differences in the metabolic profile of different cell types in the kidney. For example, cells in the inner medulla, such as those of the thin descending and ascending limbs of the loop of Henle and the medullary collecting duct, have few mitochondria and depend predominantly on glycolytic metabolism (89). In contrast, tubular cells in the cortex, such as those in proximal tubules and the thick ascending limb of the loop of Henle, are rich in mitochondria and depend predominantly on oxidative metabolism, with fatty acids, ketone bodies, and lactate being the preferred metabolic substrates (7).

There is limited information concerning the expression of the upstream AMPK kinases LKB1 and CaMKKβ in the kidney. In general, LKB1 is widely expressed in most tissues, including significant expression in the kidney (24). The LKB1-associated proteins STRADα and MO25α are also expressed in the kidney (24). LKB1 exists as both long (LKB1L) and short (LKB1S) splice variants, with the more widely expressed LKB1L (50 kDa) protein being the isoform expressed in the kidney (24). Regarding the expression of CaMKKβ, a tissue distribution study using Western blot analysis of crude lysates from different tissues found that the highest level of expression was observed in the central nervous system and that CaMKKβ protein was not detected by this method in the kidney (60). Although this study was unable to demonstrate CaMKKβ expression in the kidney, it might be that the method of detection was not sufficient to detect low levels of CaMKKβ. Further study is required to determine the roles and distribution of LKB1, CaMKKβ, and other possible AMPK kinases in the kidney.

Regulation of Sodium and Ion Transport by AMPK

While the kidney constitutes <1% of body mass, renal oxygen consumption accounts for 7% of total body oxygen consumption, and renal blood flow constitutes 20–25% of total cardiac output (72, 80). The explanation for the kidney’s large energy consumption is active tubular reabsorption of a large quantity of filtered sodium and other ions. In the 1960s, Whittam and colleagues (130) demonstrated that tubular sodium transport was closely coupled with tubular respiration, but the mechanisms linking ion transport with respiration remain incompletely defined. In recent years, AMPK has been identified as a regulator of various ion transport proteins (38). This has stimulated more general interest in the broader hypothesis that energy sensing by AMPK may be a physiologically relevant mechanism by which renal tubular cells maintain tight coupling between energy metabolism and tubular transport (16) (Fig. 2).

CFTR. The first ion transport protein to be identified as a substrate of AMPK was the CFTR Cl− channel (42). While CFTR is most well known for its functions in respiratory and gastrointestinal epithelia, transcripts for its expression are also detected throughout the nephron and it participates in Cl− secretion in the distal tubule and the inner medullary collecting duct (117). In addition, fluid secretion by CFTR appears to have an important role in the pathogenesis of cyst development in autosomal dominant polycystic kidney disease (73). The relationship between AMPK and CFTR was initially identified by a yeast two-hybrid screen, which found that the AMPK α1-subunit interacted with the C-terminal cytoplasmic tail of CFTR (42). AMPK has been shown to inhibit CFTR channel activity in both Xenopus laevis oocytes and polarized bronchial and colonic epithelial cells (40–42, 127). The mechanism involves an inhibition of the CFTR channel open probability. AMPK phosphorylates CFTR predominantly at Sε268 in the CFTR regulatory (R) domain (59, 62). We and others have shown that phosphorylation of the R domain by AMPK inhibits activation of CFTR by PKA, and tonic AMPK activity may prevent CFTR activation in the absence of cAMP agonists.
Moreover, CFTR-dependent Cl\(^{-}\) secretion is reduced by the AMPK activator phenformin and increased by the AMPK inhibitor compound 20 (13). AMPK on CFTR is physiologically relevant in vivo (63). In respiratory epithelia, AMPK also interacts with nucleoside diphosphate kinase A (NDPK-A) (124), which has been proposed to bind to CFTR. AMPK and NDPK-A may have a cooperative role in the regulation of CFTR, although the detailed mechanisms are currently under investigation. A recent study in AMPK KO mice confirmed that the effect of AMPK on CFTR is physiologically relevant in vivo (63).

AMPK activation of AMPK may limit cellular Na\(^{+}\) transport by ENaC under conditions of metabolic stress (Fig. 2).

A recent study has confirmed the role of AMPK in the regulation of ENaC in vivo (2). AMPK \(\alpha1 \rightarrow−/−\) mice have enhanced expression and activity of ENaC in the kidney, colon, and airway epithelium (2). Amiloride-sensitive fractional Na\(^{+}\) excretion was enhanced in AMPK \(\alpha1 \rightarrow−/−\) animals (2). The AMPK \(\alpha1 \rightarrow−/−\) mice also had a larger rectal potential difference and enhanced amiloride-sensitive transport in the trachea (2). Also consistent with increased ENaC activity in the AMPK \(\alpha1 \rightarrow−/−\) mice was increased urinary potassium excretion. Surprisingly, however, despite the increased renal ENaC expression, the AMPK \(\alpha1 \rightarrow−/−\) mice were observed to have reduced arterial blood pressure (2), indicating that AMPK appears to have other effects on either renal or vascular function that override the effect that increased renal ENaC expression would be predicted to have on blood pressure.

**Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransporters.** There are two distinct Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransporter (NKCC) isoforms found in epithelia, designated NKCC1 and NKCC2. NKCC1 is widely expressed and plays fundamental roles in maintaining cell volume, mediating transepithelial ion fluxes in secretory tissues, and setting the appropriate intracellular ion concentrations for excitable cells to work (33). In contrast, NKCC2 expression is restricted to the thick ascending limb and the macula densa of the kidney (33). In recent years, phosphorylation of NKCC1 and NKCC2 has been identified as an important regulatory mechanism, although details of the specific phosphorylation sites and the regulating kinases are still being elucidated (33). Other kinases that have been identified as important in regulating NKCC1 and NKCC2 include WNK1, WNK3, and the Ste-20-related kinases OSR1 and SPAK (5). On the basis of the finding that activated AMPK was detectable on the apical side of the basolateral side by the action of the Na\(^{+}\)-K\(^{+}\)-ATPase (104). Mechanisms regulating ENaC activity include synthesis, intracellular trafficking, membrane insertion and retrieval, proteolytic cleavage, and gating (9). The E3 ubiquitin-protein ligase Ned4–2 is emerging as an important locus for the regulation of ENaC activity in response to various hormonal mediators and signaling pathways. Ned4–2 interacts with the C terminus of ENaC to promote its internalization and degradation (114). We have demonstrated that activation of AMPK inhibits ENaC activity in both the X. laevis oocyte expression system and in polarized mouse cortical collecting duct (mpkCCD14) cells (16). In addition, the AMPK activators phenformin and 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR) inhibit ENaC transport in lung epithelial cells (133). In contrast to CFTR, however, AMPK does not appear to directly phosphorylate ENaC (16). The mechanism for AMPK inhibition of ENaC involves a decrease in the number of active channels at the plasma membrane (16). The inhibitory effect of AMPK on ENaC is dependent on an interaction between the ENaC β-subunit and Ned4–2 (10). AMPK is able to phosphorylate Ned4–2 both in vitro and in vivo, and activation of AMPK enhances the interaction between the ENaC β-subunit C terminus and Ned4–2 (10). This presumably leads to increased Ned4–2-dependent ENaC retrieval from the plasma membrane (10). It has also been reported that AMPK may inhibit the gating of ENaC in H441 lung epithelial cells through a phosphoinositide-dependent mechanism (81). In this way, activation of AMPK may limit cellular Na\(^{+}\) transport by ENaC under conditions of metabolic stress (Fig. 2).
membrane of the thick ascending limb and macula densa, we hypothesized that AMPK might regulate NKCC2 (29). Subsequently, we found that AMPK coimmunoprecipitated with the N-terminal cytoplasmic domain of NKCC2 and phosphorylated the N terminus of NKCC2 at position Ser126 in vitro (30). Furthermore, activation of AMPK in the macula densa cell line (MMDD1) resulted in an increase in NKCC2-Ser126 phosphorylation, suggesting that AMPK may phosphorylate NKCC2 in vivo (30). In addition, we recently found that AMPK is activated in MMDD1 cells in response to low salt, independently of osmolality (22). This effect of low salt in MMDD1 was not inhibited by bumetanide (22). Low salt-induced activation of AMPK in MMDD1 cells increased phosphorylation of both NKCC-Ser126 and ACC-Ser79 (22). When NKCC2-Ser126 was mutated to an alanine and expressed in X. laevis oocytes under isotonic conditions, cotransporter activity was markedly reduced, whereas under hypertonic conditions there was no change (30). Surprisingly, however, AMPK was not found to regulate NKCC2 transport in the X. laevis oocyte model (30). Possible explanations for this finding include endogenous AMPK expression by X. laevis oocytes causing constitutive phosphorylation, another unknown kinase that might target NKCC2-Ser126, or phosphorylation of NKCC2 by AMPK might require another protein to produce a modification of cotransporter activity.

\[ \text{Na}^+\text{K}^+-\text{ATPase} \]

The basolateral \( \text{Na}^+\text{K}^+-\text{ATPase} \) is the major active transport mechanism responsible for reabsorption of \( \text{Na}^+ \) throughout the nephron. There is presently no direct evidence, however, for a role of AMPK in the regulation of \( \text{Na}^+\text{K}^+-\text{ATPase} \) in the kidney. In contrast, there is evidence in H441 lung cells of regulation of \( \text{Na}^+\text{K}^+-\text{ATPase} \) by AMPK. Specifically, Woolhead et al. (133, 134) found that AMPK activation with phenformin and AICAR inhibited ouabain-sensitive transepithelial sodium transport. Furthermore, Vadasz et al. (126) reported in alveolar epithelial cells that elevated \( \text{PCO}_2 \) activated AMPK through CaMKKβ, leading to activation of PKCζ, thus promoting \( \text{Na}^+\text{K}^+-\text{ATPase} \) endocytosis and reduced salt and water reabsorption. A preliminary report by Seo-Mayer and colleagues (112), however, suggested that preactivation of AMPK through metformin treatment attenuated the downregulation of basolateral membrane \( \text{Na}^+\text{K}^+-\text{ATPase} \) expression following ischemia in Madin-Darby canine kidney (MDCK) cells. These results suggest that prior AMPK activation may enhance ischemic preconditioning in epithelial tissues and highlight that there may be differential effects of AMPK in epithelia depending on the setting and time course of activation (112).

Other ion transport proteins. In addition to what is known about ion channels in the kidney, regulation of ion channels by AMPK has also been proposed in the heart for voltage-gated \( \text{Na}^+ \) channels (78) and ATP-sensitive \( \text{K}^+ \) (\( K_{\text{ATP}} \)) channels (120). Specifically, Light et al. (78) found that when the human cardiac \( \text{Na}^+ \) channel hH1 and constitutively active AMPK were coexpressed in mammalian myocytes, the action potential duration was significantly prolonged. Sukhodub et al. (120) also described a role for AMPK regulation of \( K_{\text{ATP}} \) channels in a model of cardiac ischemic preconditioning. This study found that hearts overexpressing a dominant negative form of the α2-subunit of \( K_{\text{ATP}} \) were more susceptible to ischemic injury. The cytoprotective effect of AMPK was not related to the effects of ischemic preconditioning on mitochondrial membrane potential but instead to its role in preconditioning-induced shortening of the sarcolemmal action potential via \( K_{\text{ATP}} \) channels (120). A recent study also found that the calcium-activated \( K^+ \) channel KCa3.1, which is expressed at the basolateral membrane in a variety of epithelia, interacts with the AMPK γ1-subunit and is inhibited by AMPK in lung epithelial cells (61). In addition, we have shown recently that AMPK activation in the kidney-like epithelium of the epididymis inhibits PKA-induced apical membrane accumulation of the vacuolar \( \text{H}^+\text{ATPase} \) (V-ATPase) (39). Both kinases appear to directly phosphorylate one of the subunits of the V-ATPase in vitro and in cells. Very recent data also suggest a similar mode of V-ATPase regulation by AMPK in kidney collecting duct intercalated cells (34, 100). Another preliminary report suggests that the creatine transporter (SLC6A8), which is expressed at the apical membrane of proximal tubule cells and mediates reclamation of filtered creatine in the kidney (32), is inhibited by AMPK activation in mouse S3 proximal tubule cells (34) via effects on transporter plasma membrane expression (74). Finally, a preliminary report has demonstrated that the KCNQ1 potassium channel expressed in collecting duct principal cells is inhibited by AMPK, like ENaC, via a Nedd4-2-dependent mechanism (3).

### Regulation of AMPK in the Kidney

**Salt and water.** Regulation of renal AMPK activity in response to variation of salt intake and subsequent regulation of renal \( \text{Na}^+ \) transport has been hypothesized to be a possible novel mechanism of \( \text{Na}^+ \) homeostasis (29). Fraser et al. (29) have reported that AMPK activity was increased by 25% in rats receiving a high-salt diet, and this was confirmed by Western blotting for αThr172 phosphorylation. In addition, both low- and high-salt media activated AMPK in the macula densa cell line MMDD1 (22, 29). Activation of AMPK in MMDD1 cells by low salt occurred in the presence of either low \( \text{Na}^+ \) or low \( \text{Cl}^- \) and was unaffected by inhibition of NKCC2 with bumetanide (22). In addition, the antidiuretic hormone vasopressin has recently been reported to cause dephosphorylation of AMPK in MDCK clone 7 (MDCK-C7) cells (97). Since vasopressin is released from the posterior pituitary in response to water deprivation, this suggests another possible mechanism by which AMPK might contribute to fluid and electrolyte homeostasis. Taken together, the observations that AMPK activity can be regulated by both dietary salt intake and extracellular NaCl concentration and that AMPK is a regulator of various ion transport proteins suggest a physiological role for AMPK in the regulation of \( \text{Na}^+ \) and electrolyte homeostasis and its relationship to energy metabolism.

**Adiponectin.** An interesting study by Sharma et al. (113) identified a role for adiponectin and AMPK in the regulation of podocyte function and the pathogenesis of albuminuria. Adiponectin is a hormone produced by adipocytes, and the serum level is reduced in obesity. A low serum level of adiponectin in obesity is associated with albuminuria (113). Furthermore, adiponectin knockout mice exhibit increased albuminuria and fusion of podocyte foot processes (113). In cultured podocytes, adiponectin administration increased activity of AMPK, and both adiponectin and AMPK activation reduced podocyte permeability to albumin and podocyte dysfunction, as evidenced by zona occludens-1 translocation to the membrane (113).
These effects seemed to be caused by reduction of oxidative stress, as adiponectin and AMPK activation both reduced protein levels of the NADPH oxidase 4 (Nox4) in podocytes.

Cammisotto et al. (14) further studied the role of AMPK and adiponectin in glomerular function. By electron microscopy and immunogold staining, the adiponectin receptor ADIPOR1 and the catalytic AMPK subunits \( \alpha 1 \) and \( \alpha 2 \) were localized in glomeruli at the plasma membrane of endothelial, mesangial, and podocyte cells, as well as on Bowman’s capsule epithelial cells (14). Incubation of freshly isolated rat glomeruli with either adiponectin or AICAR led to the activation by phosphorylation of catalytic AMPK (14). This could suggest that activation of glomerular AMPK by adiponectin might play an important role in the control of oxidative stress and cell survival within the glomerulus.

A role for adiponectin and AMPK has also been identified by Cammisotto et al. (15) in the regulation of glycogen synthase in the distal tubule and thick ascending limb. This might be important in diabetic nephropathy, which is characterized by glycogen accumulation in distal tubular and thick ascending limb cells that eventually leads to apoptosis (8). ADIPOR1, catalytic AMPK subunits \( \alpha 1 \) and \( \alpha 2 \), and the regulatory glycogen-binding AMPK subunit \( \beta 2 \) were detected in this study by Western blots of isolated distal tubules from the rat (15). While expression levels of ADIPOR1, AMPK \( \alpha 1 \), AMPK \( \alpha 2 \), and AMPK \( \beta 2 \) were all increased in streptozotocin-treated diabetic rats, phosphorylated active AMPK (\( \alpha \text{Thr}^{72} \)) levels were strongly decreased (15). In addition, immunohistochemistry revealed the presence of ADIPOR1 on the luminal portion of distal tubules and thick ascending limb cells. The AMPK subunits \( \alpha 1 \), \( \alpha 2 \), and \( \beta 2 \) were also found in the same cells (15). In isolated distal tubules, adiponectin, acting through luminal ADIPOR1, was found to activate AMPK, which then caused inhibition of glycogen synthase, an effect that was inhibited with diabetes (15). Thus reduced AMPK activation with hyperglycemia may explain, at least in part, the accumulation of large tubular glycogen deposits in diabetic nephropathy.

**Diabetes.** It has been reported that AMPK activity is reduced in the diabetic kidney (15, 36, 69). The mechanism for this observation is unclear, but Guo et al. (36) have correlated it in streptozotocin-induced type 1 diabetes to reduced adiponectin levels. The reduced AMPK activity in the diabetic kidney does not appear to be related to altered AMP or ATP levels (69). Lee et al. (69) have associated reduced AMPK activity in the diabetic kidney with diabetes-induced renal hypertrophy. Indeed, hyperglycemia may activate the mTOR pathway by the dual effects of Akt/protein kinase B activation and AMPK inhibition, thereby contributing to basement membrane thickening and mesangial matrix accumulation (77). Increased protein synthesis with reduced AMPK activity can also be explained by AMPK regulation of eukaryotic elongation factor 2 kinase (eEF2 kinase) (12), which in turn regulates the elongation phase of mRNA translation by phosphorylation of eukaryotic elongation factor 2 (eEF2) (48, 70). In cultured glomerular epithelial cells, the AMPK activators metformin and AICAR increased AMPK phosphorylation, inhibited high-glucose stimulation of protein synthesis, and prevented high glucose-induced changes in phosphorylation of 4E binding protein 1 and eEF2 (69). In addition, expression of kinase-inactive AMPK further increased high glucose-induced protein synthesis. Furthermore, renal hypertrophy in rats with streptozotocin-induced type 1 diabetes was associated with reduced AMPK phosphorylation and increased mTOR activity. Reduced AMPK activity in the diabetic kidney has also been linked to increased triglyceride accumulation because of reduced inhibitory phosphorylation of acetyl-CoA carboxylase (36). The increased triglyceride accumulation in the diabetic kidney appears to increase expression of connective tissue growth factor, which is another mechanism that contributes to diabetes-induced renal hypertrophy (36). In addition, as described above, reduced AMPK activity in the diabetic kidney has been associated with increased renal tubular glycogen accumulation (15).

Another recent study by Lee et al. (70) also found that high glucose suppressed AMPK activity in cultured glomerular epithelial cells. In this study, hyperglycemia also increased the acetylation and reduced the activity of LKB1. Interestingly, the effects of hyperglycemia on both LKB1 and AMPK were reversed by addition of resveratrol, which is a polyphenol compound present in grapes and green tea that has been linked with increased longevity and amelioration of diabetes and the metabolic syndrome. The reduced acetylation of LKB1 in the presence of resveratrol was not dependent on silent information regulator 1 (SIRT1). The effect of resveratrol on AMPK also prevented the increased protein synthesis observed with hyperglycemia by correcting phosphorylation changes seen in eIF4E, eEF2, eEF2 kinase, and p70S6 kinase (70).

In addition to the effects of glycemia on AMPK in the kidney, both metformin and the thiazolidinediones (TZDs), which are currently used treatments for diabetes, activate AMPK independently of serum glucose (31). In diabetic rats, metformin increased renal AMPK phosphorylation, which then reversed mTOR activation and inhibited renal hypertrophy without affecting hyperglycemia (69). The TZD pioglitazone was observed to activate AMPK in a renal tubular cell line due to decreased mitochondrial membrane potential (125), but whether this observation contributes to the well-described effects of TZDs on kidney function is unknown (110).

**Ischemia.** Given that AMPK is activated in response to an increase in cellular AMP/ATP ratio, it is not surprising that acute renal ischemia is a potent activator of AMPK. In fact, AMPK is activated within 1 min of the onset of acute ischemia, and by 5 min it is dramatically activated (95). By immunohistochemistry, the predominant site of AMPK activation in response to brief acute renal ischemia was found to be cortical tubules (95). The functional significance of AMPK activation in acute renal ischemia has, however, not yet been adequately studied. Specifically, it remains to be determined whether the net effect is beneficial, harmful, or neither. Lin et al. (79) have reported that combination therapy with the AMPK activator AICAR and the antioxidant N-acetylcysteine attenuates ischemia-reperfusion injury in a canine model of autologous renal transplantation, which suggests that AMPK pretreatment might have a protective role in renal ischemia-reperfusion injury (79). This needs to be interpreted with caution, however, as the relative contributions of AICAR and N-acetylcysteine to this result are unclear. Also, AICAR can cause biological effects that are independent of its effects on AMPK. It is also unknown what the downstream targets are for AMPK in the ischemic kidney, and they could even differ from what has been described in other organs. For example, in contrast to the ischemic heart (19), in the ischemic kidney AMPK does not phosphorylate endothelial nitric oxide synthase (eNOS) (95). Furthermore, the upstream AMPK
kinase(s) required for activation of AMPK in the ischemic kidney have not yet been identified.

In contrast to this paucity of knowledge of AMPK in the kidney, there is now a significant body of literature describing an important role for AMPK in response to acute myocardial ischemia (6, 17, 66, 84, 91, 105, 108, 137). In the heart, AMPK is activated rapidly during ischemia and in most studies the net effect of this activation appears to be beneficial (66, 84, 105). This view of a beneficial role of AMPK in myocardial ischemia has, however, been challenged by Lopaschuk’s group (28), who have observed that in some situations activation of AMPK in myocardial ischemia is deleterious. The role of AMPK might also differ between ischemia and reperfusion. For example, in AMPK α2 knockout mice subject to no-flow or low-flow myocardial ischemia, there is a more rapid onset of left ventricular dysfunction but there is no difference in the recovery of contractile function after reperfusion (17, 140). It also appears that the functional effect of AMPK in response to organ ischemia may differ between different organs. For example, in contrast to its apparent protective role in myocardial ischemia, activation of AMPK is reported to be deleterious in acute stroke (75, 87). Thus it is reasonable to propose that the role and consequences of AMPK activation in ischemic injury could depend on the nature of the ischemic insult, both the timing and severity of AMPK activation, and the tissue involved.

Ischemia-associated downregulation of various epithelial transport proteins may help prevent the dissipation of transmembrane cellular ionic gradients that are important for normal cellular functioning and survival (38). Whereas ischemia activates AMPK in the kidney (95) and AMPK activation, like ischemia, induces the acute downregulation of various transport proteins (16, 30, 34, 42, 74), it is reasonable to propose that the ischemia-induced inhibition of epithelial transport is mediated by AMPK. As such, this acute inhibition of ion transport proteins by AMPK may represent an adaptive response by limiting the need for active transport via the sodium pump to maintain transmembrane ionic gradients. It is also possible that ischemia-induced activation of AMPK might help explain the increased fractional excretion of sodium that is observed (59). In the young rats, administration of D-[1-14C]glucose to rats subjected to no-flow or low-flow ischemia has been shown to decrease the fractional excretion of sodium (59). In the older animals, however, there is no difference in the fractional excretion of sodium that is observed (54). Percy et al. (102) also found that renal phosphorylation or expression with menadione was increased with aging (54). Jin et al. (54) found that the phosphorylation of AMPK in the kidneys of aged rats (24 mo) compared with young rats (2 mo) there was a nearly threefold increase in the expression of active phosphorylated AMPK (αThr172), despite the fact that total AMPK α1 expression in the older animals was reduced by ~30%. In the young rats, administration of menadione, which is an inducer of oxidative stress, caused a twofold increase in the expression of active (αThr172 phosphorylated) AMPK, whereas in the aged rats no change in AMPK phosphorylation or expression with menadione was observed (54). Percy et al. (102) also found that renal phosphorylation of AMPK (αThr172) expression was increased with aging in Wistar rats, but not in spontaneously hypertensive and Wistar-Kyoto rats. The explanation for the increased renal AMPK activity observed with aging in these studies is unclear, although Jin et al. (55) speculated that it could be related to the fact that in aged animals under various stresses there are decreased intracellular ATP levels from decreased cellular energy metabolism and accelerated depletion of ATP. Neither of these two studies was able to establish the role of increased AMPK activity in the aging kidney, and whether it contributes to reduced kidney function with age or is a protective response.

Inflammation. Activation of AMPK has been described to have anti-inflammatory effects (57, 106, 141). For example, the AMPK activator AICAR inhibits TNF-α- and IL-1β-induced NF-κB reporter gene expression dose dependently in immune cells and inducible nitric oxide synthase and cyclooxygenase-2 (COX-2) expression in stimulated macrophages (106). In addition, activators of AMPK inhibit chemotaxis in the monocyte-like cell line U937 (57). The only study to date that specifically examines the role of AMPK in inflammation in the kidney is by Peairs at al. (101) in mesangial cells derived from the MRL/lpr mouse that develops a form of renal disease similar to lupus nephritis. This study found that activation of AMPK by AICAR profoundly inhibited lipopolysaccharide- and IFN-γ-stimulated production of the proinflammatory molecules nitric oxide synthase, COX-2, and IL-6. AICAR did not, however, cause NF-κB (p65) nuclear translocation in MRL/lpr mesangial cells (101). Further evidence for cross talk between the AMPK pathway and the immune system comes from the finding that macrophage migration-inhibitory factor (MIF) activates AMPK in the heart (91). While the kidney also has significant endogenous MIF expression (67), it is yet to be determined whether MIF can also regulate AMPK in the kidney. Further studies are required to examine the role of AMPK in inflammatory diseases of the kidney.

Endothelial function. A growing body of evidence for a variety of important roles for AMPK in the regulation of endothelial cell function has recently been reviewed in detail (27). Activation of AMPK in endothelial cells has been reported in response to multiple and diverse stimuli, including shear stress (20), ATP depletion (95, 144), hormones such as adiponectin (18, 20, 99), bradykinin (96) and thrombin (116), the drugs metformin (143) and atorvastatin (20), and the free radical peroxynitrite (144). Activation of AMPK in endothelium has been implicated in the regulation of fatty acid oxidation (23, 96), nitric oxide production (94), inflammation (13), and angiogenesis (99). Overall, the effect of AMPK in endothelium has been proposed to be antiatherogenic and to potentially improve the endothelial dysfunction observed with diabetes and the metabolic syndrome (71, 85). It is important to acknowledge, however, that while Cammissoto et al. (14) have demonstrated AMPK expression in glomerular endothelial cells, to date the specific roles of endothelial AMPK in the kidney have not been defined.

Glucogenesis. In addition to the more-well-known role of the liver, the renal cortex is also a significant site of glucogenesis. For example, it has been reported that in the postabsorptive phase renal glucose release approaches 20% of all glucose released in the circulation (90). The site of renal glucogenesis is the proximal tubule (35). While activation of AMPK has been clearly demonstrated to suppress glucogenesis in the liver (64), a role of AMPK in the regulation of renal glucogenesis has not been studied. In the liver, activation of AMPK promotes phosphorylation of the transducer of regulated CREB activity 2 (TORC2), which then reduces the ability of TORC2 to transactivate glucogenic genes such as phosphoenolpyruvate carboxykinase (64). This appears to be the mechanism by which AMPK-activating drugs such as metformin suppresses hepatic glucogenesis (142). Further
studies are required to determine whether AMPK has a role in the regulation of renal gluconeogenesis and whether this contributes to the antidiabetic effects of AMPK activators.

Summary and Future Directions

While it has been known for over a decade that the metabolic sensor AMPK is abundantly expressed in the kidney (118), an understanding of its roles in the kidney is now beginning to emerge. AMPK is potentially an important regulator of energy metabolism in the kidney. For example, AMPK is a key regulator of lipid metabolism, and in the kidney fatty acids are an important energy source (7). Significant fatty acid and triglyceride synthesis occurs in the kidney (128). Glucose is also an important metabolic substrate for the kidney (129).

AMPK is known to be an important regulator of multiple aspects of glucose metabolism, including glycolysis, glucose uptake, glycogen synthesis, and gluconeogenesis (43). Present knowledge of AMPK biology in the kidney is significantly less advanced than that for other organs such as muscle, adipose tissue, liver, and heart. For example, little is known about the roles of upstream AMPK kinases such as LKB1 and CaMKβ in the regulation of AMPK in the kidney. It is apparent, however, that AMPK has a variety of important roles in the kidney in both physiology and disease. Regarding physiology, most interest has focused on the role of AMPK in the regulation of ion transport by transport proteins such as CFTR (42), ENaC (16), NKCC2 (30), and the V-ATPase (39). This work potentially throws fresh mechanistic light on the hypothesis of Whittam (130), who described the close coupling that exists between cellular ionic transport and respiration almost a half-century ago. Recent important studies showing a role for adiponectin in the regulation of AMPK in the kidney (14, 15, 113) suggest that systemic factors could be as important as local factors in regulating renal AMPK function. Surprisingly, even though the kidney is an important contributor to whole body glucose homeostasis (83), there are as yet no studies examining the role of AMPK in renal glucose metabolism. Regarding kidney diseases, an emerging area of interest is the role of AMPK in autosomal dominant polycystic kidney disease (86, 122). Other kidney diseases where AMPK is of increasing interest include acute renal ischemia (95) and diabetic nephropathy (15, 69). Further studies are required to advance our current understanding of the roles of AMPK in renal physiology and disease and, specifically, to identify the specific protein substrates targeted by AMPK in the kidney.

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


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