Regulation of the energy sensor AMP-activated protein kinase in the kidney by dietary salt intake and osmolality

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Fraser, Scott, Peter Mount, Rebecca Hill, Vicki Leviotis, Frosa Katsis, David Stapleton, Bruce E. Kemp, and David A. Power. Regulation of the energy sensor AMP-activated protein kinase in the kidney by dietary salt intake and osmolality. Am J Physiol Renal Physiol 288: F578–F586, 2005. First published November 9, 2004; doi:10.1152/ajprenal.00190.2004.—The AMP-activated protein kinase (AMPK) is a key controller of cellular energy metabolism. We studied its expression and regulation by salt handling in the kidney. Immunoprecipitation and Western blots of protein lysates from whole rat kidney using subunit-specific antibodies showed that the α-catalytic subunit is expressed in the kidney, associated with the β- and either γ1- or γ2-subunits. Activated AMPK, detected by immunohistochemical staining for phospho-Thr172 AMPK (pThr172), was expressed on the apical surface of the cortical thick ascending limb of the loop of Henle, including the macula densa, and some parts of the distal convoluted tubule. Activated AMPK was also expressed on the basolateral surface of the cortical and medullary collecting ducts as well as some portions of the distal convoluted tubules. AMPK activity was increased by 25% in animals receiving a high-salt diet, and this was confirmed by Western blotting for pThr172. Low-salt diets were associated with reduced levels of the α-subunit of AMPK, which was highly phosphorylated on Thr172. Surprisingly, both low- and high-salt media transiently activated AMPK in the macula densa cell line MMD1, an effect due to changes in osmolality, rather than Na+ or Cl− concentration. This study, therefore, demonstrates regulation of AMPK by both a high- and a low-salt intake in vivo and suggests a role for the kinase in the response to changes in osmolality within the kidney.

Materials and Methods

Animal models. The Animal Ethics Committee of Austin Health approved all animal procedures. Male Sprague-Dawley rats weighing 200–250 g, purchased from the Western Australian Research Facility, were fed a diet of high salt (8.95% NaCl; sodium toxicity diet, ICN, Costa Mesa, CA), low salt (0% NaCl; sodium-deficient diet, ICN), or standard rat chow (0.5% NaCl; Ralid Agriproducts, Pakenham, Victoria, Australia) for 2 wk. At the end of the experiment, the animals were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). A midline incision was made, and the left kidney was freeze-clamped in situ to provide protein for biochemical analysis. For immunohistochemistry, kidneys were biopsied and immediately fixed by immersion in 4% paraformaldehyde. To confirm the immunohistochemical findings, perfusion-fixed kidneys from Sprague-Dawley rats, the generous gift of Dr. Wilkinson-Berk, Department of Anatomy, University of Melbourne, were also used.

Antibodies. As previously described (10, 19, 47), polyclonal antibodies were raised to nonconserved regions of the AMPK isoforms α1 (373–390, CARHTLDLELPQKSKHQG) and α2 (490–516, CASAGLHRPRSSVDSTAEHLSLGG). Anti-AMPK β-antibodies were raised against the peptide CSPPPGYPQPYISKPE-NH2 (181–197; this antibody is now recognized to detect both β1 and β2, which can be distinguished by their apparent molecular mass. AMPK γ1-antibodies were raised against the peptide CQLALVTGGEKPK-PYHH (319–331), whereas AMPK γ2-antibodies were raised against the peptide CTPAGAKQETETE-COOH (555–569) and γ3 against the peptide AESTGLETTPKKT (59–75). All antibodies were highly phosphorylated on Thr172. Surprisingly, both low- and high-salt diets were associated with reduced levels of the α-subunit of AMPK, which was highly phosphorylated on Thr172. Surprisingly, both low- and high-salt media transiently activated AMPK in the macula densa cell line MMD1, an effect due to changes in osmolality, rather than Na+ or Cl− concentration. This study, therefore, demonstrates regulation of AMPK by both a high- and a low-salt intake in vivo and suggests a role for the kinase in the response to changes in osmolality within the kidney.

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Because the major energy-requiring process in the kidney is sodium transport (31), we hypothesized that AMPK is likely to be activated by salt loading and to phosphorylate molecules important in the response to changes in dietary salt. In this study, therefore, we determined the distribution of the active form of AMPK in normal rat kidney and demonstrated that salt intake regulates AMPK.
antigen affinity purified and tested for reactivity by ELISA with the immunizing peptide and for specificity by Western blotting using purified AMPK. A further antibody was raised against the activated α1-subunit with the phosphopeptide AMPK α1 KDGEFLRPrTSGC-SPNY (165–179). The antibody was purified from sera using the corresponding phosphopeptide affinity column after preclearing with a dephospho affinity column specific for the dephospho version of the immunizing peptide (47). Affinity-purified rabbit polyclonal antibodies were produced against the phosphorylation site phospho-nNOS-Ser1412 as previously described (11). A monoclonal mouse antibody against nNOS (N31020) was purchased from BD Transduction Laboratories (Lexington, KY).

**Immunohistochemistry.** Tissue was immersion fixed in 4% paraformaldehyde (BDH, Poole, UK), processed, and embedded in paraffin, as previously described (29). Briefly, 4-μm-thick paraffin sections were incubated overnight with the anti-p-Thr172 phosphopeptide antibody (5 μg/ml) at 4°C. Rabbit IgG was detected using the rabbit peroxidase-antiperoxidase (PAP) technique, comprising a goat anti-rabbit Ig (Dako, Carpinteria, CA) diluted at 1/100, followed by rabbit PAP (Dako) diluted 1/100. Peroxidase labeling was revealed using the liquid DAB substrate-choromagen system (Dako). Sections were counterstained with hematoxylin.

**Localization of staining using tubular markers.** To determine the location of tubular staining of activated AMPK, double staining confocal microscopy was performed with FITC-conjugated tubular markers and the AMPK anti-p-Thr172 phosphopeptide antibody (5 μg/ml). Paraffin sections were dewaxed and then quenched with 150 mM glycine for 20 min. Sections were permeabilized with 0.3% Triton X-100/0.025% CHAPS/PBS and nonspecific staining was blocked by the addition of 5% BSA. Staining was performed overnight and rabbit IgG was detected using anti-rabbit antibody conjugated to Alexa 594. To study the expression of activated AMPK in distal tubules and collecting ducts (CD), FITC-conjugated peanut agglutinin (Sigma, St. Louis, MO) was incubated overnight (final concentration 5 μg/ml). To stain proximal tubules, FITC-conjugated Phaseolus vulgaris erythroagglutinin (Sigma; PHA-E) was used (final concentration 5 μg/ml). Sections were then studied by confocal laser-scanning microscopy (Leica Microsystems, Heidelberg, Germany).

**AMPK activity assay.** AMPK activity was measured by the extent to which the sample phosphorylated the AMPK consensus sequence of the ADR-1 peptide (LKKTTLRPSFSFSAQ-ame), as previously described (15, 35). Briefly, AMPK was immunoprecipitated and incubated at 30°C with 100 μM ADR-1 peptide in reaction buffer (50 mM HEPES, pH 7.5, 10 mM MgCl2, 5% glycerol, 1 mM DTT, 0.05% Triton X-100) with 250 μM [γ-32P]ATP (500 cpm/pmol) for 8 min. After the reaction, 25 μl of reaction mixture were spotted onto the phosphocellulose P81 paper and extensively washed with phosphoric acid. The radioactivity on the filter paper was measured by scintillation counting, and AMPK activity was expressed as picomoles of 32P incorporation into the peptide per minute per microgram of protein.

**Cell culture.** The murine MD cell line MMDD1 (55) was very kindly provided by Dr. J. Schnermann (National Institutes of Health, Bethesda, MD) and grown in DMEM media supplemented with 10% FCS until confluence. Quiescent MMDD1 cells were then exposed to media of low-, normal-, or high-salt content with or without osmolality controls (Table 1) as previously described (13). Cells were incubated in lysis buffer for 5 min on ice and then centrifuged at 18,000 g for 5 min at 4°C and the resulting pellets were discarded. Protein concentration was then determined by Bradford assay using a commercial protein assay solution (Bio-Rad), and the lysates were used for immunoprecipitation.

**Statistics.** Statistics were performed using Instat Version 3.05 (GraphPad Software, San Diego, CA). Data are presented as means ± SD. Unless stated, data were analyzed by ANOVA using Bonferroni’s test of multiple comparisons. *P* values <0.05 were considered significant.

**RESULTS**

**AMPK subunit expression in the kidney.** The expression of AMPK subunits in the kidney was determined by immunoprecipitation experiments using antibodies against the α1- and α2-, γ1-, and γ2-subunits followed by Western blots using antibodies against all the known AMPK subunit isoforms (Fig. 1).

**Table 1. Composition of low-, normal-, and high-salt media (modified from Ref. 13)**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Composition, mM</th>
<th>[Na+], mmol</th>
<th>Osmolality Calculated/Actual*, mosmol/kgH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low salt</td>
<td>25 NaHCO3</td>
<td>0.96 NaH2PO4</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>0.24 Na2HPO4</td>
<td>0.24 KCl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.2 MgSO4</td>
<td>1.0 CaCl2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.5 glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal salt</td>
<td>100 mM NaCl</td>
<td>126</td>
<td>274/264</td>
</tr>
<tr>
<td>High salt</td>
<td>200 mM NaCl</td>
<td>226</td>
<td>474/462</td>
</tr>
<tr>
<td>Low salt, normal osmolality</td>
<td>200 mM mannitol added</td>
<td>26</td>
<td>274/261</td>
</tr>
<tr>
<td>Normal salt, high osmolality</td>
<td>100 mM NaCl</td>
<td>126</td>
<td>474/464</td>
</tr>
<tr>
<td></td>
<td>200 mM mannitol added</td>
<td></td>
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</table>

*Osmolality of the solutions was tested using the Advanced Micro-Osmometer model 3300 to give “Actual” osmolalities. The osmolalities calculated from sample composition are also given as “Calculated” osmolalities.
pattern of subunit expression in the kidney was found to be different to that in heart, liver, and skeletal muscle. In the kidney, the predominant isofrom of the catalytic subunit was α1 (Fig. 1, A–D), whereas the α2-subunit was found to be expressed either as a faint band (Fig. 1B) or to be undetectable (Fig. 1, C and D). The 30-kDa β2-subunit was detected following immunoprecipitation with antibodies against α1-, α2-, γ1-, and γ2-subunits (Fig. 1). In contrast to the other tissues examined, the 36-kDa β1-subunit was generally not detected in kidney lysates (Fig. 1), although some other blots did demonstrate a faint 36-kDa β1 band consistent with a low level of expression (data not shown). The γ1- and γ2-subunits were found to coprecipitate with both α-subunits (Fig. 1, A and B). This was confirmed by Western blots following immunoprecipitation with antibodies against the γ1- and γ2-subunits (Fig. 1, C and D). The skeletal muscle-specific γ2-subunit was not detected following immunoprecipitation with antibodies against the α1-, α2-, and γ3-subunits (data not shown). The γ2-subunit has been described to exist as a long (63 kDa) and a short form (37 kDa), with the long form appearing predominant in the kidney at a mRNA level (14, 34). Surprisingly, we were unable to detect the 63-kDa long form of γ2 by Western blot. However, a 37-kDa species consistent with the short form of γ2 was detected in the kidney and other rat tissues. In fact, a double band close to 37 kDa was observed in the γ2 blots. Whether this represents some form of posttranslational modification or an artifact caused by proteolysis is unclear.

**Distribution of activated AMPK in the kidney.** To determine the location of activated AMPK, a polyclonal antibody against AMPK phosphorylated at the Thr172 site (pThr172) in the activation loop of the α1-subunits and α2-subunits was used. Immunohistochemistry revealed labeling restricted to the apical or basolateral surface of a population of tubules (Fig. 2). In the cortex, there was apical staining of approximately half of the TAL (Fig. 2, A and F), demonstrated by staining of sequential sections with anti-Tamm-Horsfall antibody (not shown). The MD, identified by staining of sequential sections with nNOS antibody, also expressed activated AMPK (Fig. 2, E and G). Cells of the proximal convoluted tubule, identified by immunofluorescence microscopy using FITC-conjugated PHA, were negative (Fig. 3C). There was weak apical staining in some cells of the distal convoluted tubule (Fig. 3B), identified using the FITC-conjugated lectin PNA and immunofluorescence microscopy. CD in both the cortex and medulla showed basolateral staining (Fig. 2, B and D), although this was variable and sometimes weak. These tubules were identified using FITC-conjugated PNA (Fig. 3, A, D, and E). As a control for the specificity of staining, the antibody was preincubated with the immobilizing phosphopeptide or the phosphopeptide, both coupled to SulfoLink resin. Preincubation with the specific phosphopeptide removed all staining, whereas the phosphopeptide had no effect (data not shown). Immunohistochemical staining of perfusion-fixed sections was identical to the immersion-fixed sections (data not shown).

**Regulation of AMPK activity by salt in vivo.** Because tubular salt reabsorption is a major determinant of energy consumption in the kidney, we investigated the effect of dietary salt concentration on AMPK activity. Rats were fed a low-, normal-, or high-salt diet and AMPK activity was determined by activity assay and detection of p-Thr172. There was a 25% increase in AMPK activity in rats fed a high-salt diet (P < 0.001) but no change in the low-salt group (Fig. 4). This was confirmed by detection of p-Thr172 (Fig. 5, A–D). There was no apparent change in the distribution of p-Thr172 by immunofluorescence microscopy in rats receiving a low- or a high-salt diet (data not shown). Although there was no change in Thr172 expression in rats fed a low-salt diet (Fig. 5C), these kidneys had reduced α1-subunit expression (Fig. 5B). When corrected for the low level of α1, the proportion of AMPK phosphorylated on the activating p-Thr172 site in these rats was higher than in the normal rats (Fig. 5D).

**Regulation of AMPK activity by salt and osmolality in vitro.** Incubation of cells in media of different composition has been used as a surrogate for changes in tubular fluid (13, 55). To determine the effect of salt concentrations on AMPK activity, therefore, the MD cell line MMD1 (55) was incubated with low-, normal-, or high-salt media (Table 1) for periods up to 24 h. The AMPK subunit distribution of MMD1 was first determined by immunoprecipitation and Western blot analysis and found to be similar but not identical to that of whole kidney. The major subunits present were α1, β1, γ1, and γ2 (Fig. 6), but there was no detectable β2, as in the whole kidney. The cells were incubated in low-, normal-, or high-salt media to determine the effect of salt on AMPK activation. There was a rapid (5 min) increase in AMPK activity in the low-salt medium, which peaked at 15 min (Fig. 7B), was maintained to 3 h, and returned to baseline by 24 h (Fig. 7D). This was confirmed by changes in p-Thr172, although there was a con-
continued slight increase at 24 h when AMPK activity had returned to baseline (Fig. 7, A and C). The high-salt medium also showed an increase in AMPK activity, but not until 30 min (Fig. 7B) and had returned to baseline by 3 h (Fig. 7D). This was confirmed by increases in p-Thr172, with continued slight elevation at 3 h when AMPK activity had returned to baseline (Fig. 7, A and C). In the high-salt group, there was an apparent dissociation between p-Thr172 and AMPK activity at 24 h. This was inconsistent and associated with evidence of cellular toxicity.

To determine whether the effect of salt concentration was mediated by changes in osmolality, osmotic controls were prepared using mannitol to restore the osmolality of the low-salt medium while the reduced salt concentration was maintained (Fig. 8, A and B) or to increase the osmolality of normal salt-containing medium to that of the high-salt medium (Fig. 9, A and B). These controls showed that restoration of normal osmolality prevented activation of AMPK associated with low-salt media, as determined by the presence of p-Thr172 (Fig. 8A) and AMPK activity assays (Fig. 8B). Moreover, increasing...
osmolality in the normal-salt medium to the level of the high-salt medium produced an equivalent increase in p-Thr172 and AMPK activity (Fig. 9, A and B). These data demonstrate that AMPK activation in MMDD1 cells is mediated by changes in osmolality and not salt concentration per se.

**DISCUSSION**

To determine the AMPK subunits present in the kidney, lysates from kidney, heart, liver, and skeletal muscle were immunoprecipitated with antibodies against AMPK subunits and then immunoblotted. The kidney was unique when compared with liver, heart, and skeletal muscle, in the virtual absence of the α2-catalytic subunit and the β1-regulatory subunit. We next attempted to determine whether there was any region of the kidney that expressed activated AMPK. Immunohistochemical and immunofluorescence staining of normal rat kidneys with an antibody against pThr172 showed that the cortical TAL and MD strongly expressed activated AMPK on the apical surface. There was also some staining on the basolateral surface of the CD, although this was not present in all kidneys.

Fig. 3. Immunolocalization of activated AMPK using lectins and laser-scanning confocal microscopy. C: double staining against activated AMPK (red fluorescence) and the proximal tubule cell marker PHA-E (green fluorescence). Activated AMPK tubules did not colocalize with proximal tubules. A, B, D, and E: double staining with anti-p-Thr172 Ab (red fluorescence) and the lectin PNA (green fluorescence), which has been shown to stain the apical surface of distal tubules including the colleting ducts. F: section incubated with an irrelevant Ab as a negative control. B and E: scale bars = 10 μm. A, C, D, and F: scale bars = 50 μm.

Fig. 4. AMPK activation in rats following 2 wk of a high-, normal-, or low-salt diet. Lysates immunoprecipitated with anti-α1 AMPK Ab were subjected to AMPK activity assay. Results are shown as means ± SD of 5 separate assays. *P < 0.001.
The unexpected ability of both high- and low-salt diets to modify AMPK activation was supported by studies performed in the MD cell line MMDD1. MMDD1 was used in these studies because the effect of salt concentration and tubular flows has been most well studied in the MD. In that location, the feedback signal for tubuloglomerular feedback (TGF) appears to be tubular Cl⁻/H⁺ concentration (43, 44). In MMDD1 cells, however, osmolality, whether high or low, was the stimulus for AMPK activation. Although this might argue against a role for AMPK, there is some evidence that osmolality can also mediate TGF (6, 7, 21).

Although the data from the cell culture studies are supportive of the in vivo data, there are several caveats that must be made, beyond the usual reservations with cell culture as a model for events occurring in the whole animal. First, the time courses over which the observations were made clearly differ, with animals exposed to high-salt diets for 2 wk compared with only 24 h for cells in culture. In vivo, moreover, the tubules exposed to high salt might vary over time, as there was only about half of the TAL that showed detectable staining for p-Thr<sup>172</sup> at any one time. Interestingly, the pattern of staining for p-Thr<sup>172</sup> was the same in high-, normal-, and low-salt groups. Second, exposure of cultured cells to hypertonic or hypotonic stimuli is likely leading to changes in cell shape, which could stimulate AMPK. There is no evidence that tubular cells in vivo undergo similar changes in cell volume related to the more gradual changes in osmolality of the tubular fluid that they are likely to encounter.

High osmolality is a well-known activator of AMPK (25), but this is the first report of low osmolality causing AMPK activation. The ability of the two divergent stimuli, low and high osmolality, to transiently activate AMPK suggests the likelihood of different renal AMPK substrates, but their identity is currently unknown. Two other kinases are also regulated by dietary salt intake. Both erk1/2 and p38 MAPK are activated by increased dietary salt intake (57), but low-salt diets also activate p38 MAPK (13). The apparently paradoxical observation with AMPK, therefore, has a counterpart in regulation of p38 MAPK.

The role of AMPK in the kidney is unknown, but the distribution of the activated kinase suggests several potential substrates. nNOS and eNOS are known substrates for AMPK.

Fig. 5. Kidneys from rats on low-, normal-, or high-salt diets were harvested and lysates were prepared for immunoprecipitation with anti-α₁ AMPK Ab followed by Western blotting with anti-p-Thr<sup>172</sup> Ab. Representative data (A) and densitometry from 5 independent experiments (means ± SD) are shown for blots probed with anti-α₁ subunit (B), anti-pThr<sup>172</sup> (C), and the ratio of the densitometry of anti-pThr<sup>172</sup>/α₁ AMPK (D). *P < 0.05. **P < 0.001.

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Fig. 6. AMPK subunits in MMDD1 cells, determined by immunoprecipitation with anti-α₁ or -α₂ Ab (IP Ab) followed by Western blotting with antibodies against each of the AMPK subunits except γ₁ (shown on the right).
nNOS is restricted in its immunohistochemical distribution to the MD (2, 50), although its mRNA is more widely distributed, especially in the medulla (53). Immunohistochemical staining for pThr^{172} AMPK demonstrated areas of codistributed in this study with nNOS in the MD. This may be relevant because AMPK is one of only two kinases known to phosphorylate nNOS (11, 26). NO produced by nNOS is important in resetting TGF and is probably important in excretion of a salt load (16, 42). If AMPK were to phosphorylate nNOS in vivo, then it could contribute to control of NO release from these cells, together with other stimuli such as intracellular Ca, which is reported to increase during salt loading (4, 40). Another potential role is in regulation of ion channels or sodium cotransporters, and preliminary data from laboratories including our own have suggested that

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**Fig. 7.** Activation of AMPK in MMDD1 cells by incubation in low, normal, or high salt. A and C: MMDD1 cells were incubated in low-, normal-, or high-salt media (as outlined in Table 1). Cell lysates were then immunoprecipitated with anti-α₁ AMPK Ab and immunoblotted with anti-p-Thr^{172} Ab or anti-α₁ Ab as a loading control. Representative data are shown from 3 experiments. B and D: cell lysates were immunoprecipitated as before and then assayed for AMPK activity by AMPK activity assay. Data pooled from 3 experiments are shown as means ± SD. *P < 0.05, **P < 0.001. ‡Cellular toxicity, evidenced by some lifting of cells from the plate, was observed at 24 h in cells exposed to high-salt media.

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**Fig. 8.** Osmolality (Osm) control for low-salt media. MMDD1 cells were incubated with low- or normal-salt media, and then the osmolality was corrected to normal with mannitol or left alone (Table 1). AMPK activity was assessed by immunoprecipitation and immunoblotting with anti-p-Thr^{172} (A) or AMPK activity assay (B). Data pooled from 3 experiments are shown as means ± SD. *P < 0.05.

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**Fig. 9.** Osmolality control for high-salt media. MMDD1 cells were incubated with normal- or high-salt media, and then the osmolality was maintained as normal or increased to high with mannitol (Table 1). AMPK activity was assessed by immunoprecipitation and immunoblotting with anti-p-Thr^{172} (A) or AMPK activity assay (B). Data pooled from 3 experiments are shown as means ± SD. *P < 0.05, **P < 0.001.
both ENaC (22) and the Na-K-2Cl (NKCC) cotransporter could be regulated by AMPK (17).

If AMPK has a role in regulation of MD function, it would provide another level of control of TGF by adenine nucleotides such as ATP and AMP. Much interest has centered recently on the regulation of TGF by ATP and the adenine nucleotide derivative adenosine. Adenosine receptor (A1AR) knockout mice demonstrate a complete absence of TGF responses to saturating increases in MD NaCl (8, 48) and adenosine receptor antagonists also inhibit TGF (49). The source of adenosine in the region of the MD is unclear; initially, it was considered that adenosine was generated in MD cells by dephosphorylation of ATP when a rise in transcellular NaCl transport increased cellular energy demand (38). Recent data suggest that cells of the MD release ATP from their basolateral surface in response to high-salt loads (5, 32). Extracellular ATP might then be converted to adenosine by ecto-5′-nucleotidase. AMPK is the most important of a family of kinases and other molecules that are regulated, in contrast, by changes in the intracellular level of the adenine nucleotides ATP and AMP (30, 33, 45).

If ATP is lost through a specific release mechanism on the basolateral surface of cells when tubular NaCl and flows are high, then intracellular levels may fall, so disturbing the AMP/ATP ratio. In addition, salt reabsorption is the major energy-consuming process in the kidney (31), generally through the action of a basolateral Na-K-ATPase. In the MD, there is very little Na-K-ATPase and most of the sodium extrusion from the cell appears to be due to an apical H-K-ATPase that also transports Na (reviewed in Ref. 4). The net effect, however, might be similar in that high-salt loads would lead to greater activity of the Na-H/K-ATPase and greater production of AMP at the expense of ATP. AMPK activation, therefore, could occur when tubular flows are high through release of ATP into the extracellular environment and generation of AMP by the apical Na-H/K-ATPase. This proposition would be strengthened by direct measurements of AMP levels within cells of the MD during salt loading.

Hallows et al. (23) previously suggested that AMPK could link the metabolic state of a cell and its handling of ion transport. This study supports that prediction, showing that AMPK is activated in the kidney by salt intake. Activation of AMPK in cells of the MD also suggests a potential role for intracellular adenine nucleotides in control of TGF. This complements emerging evidence for the role of extracellular ATP and adenosine in TGF and is an exciting possibility for future study.

GRANTS

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