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Inhibition of the heterotetrameric K^+ channel KCNQ1/KCNE1 by the AMP-activated protein kinase

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Abstract

The heterotetrameric K^+-channel KCNQ1/KCNE1 is expressed in heart, skeletal muscle, liver and several epithelia including the renal proximal tubule. In the heart, it contributes to the repolarization of cardiomyocytes. The repolarization is impaired in ischemia. Ischemia stimulates the AMP-activated protein kinase (AMPK), a serine/threonine kinase, sensing energy depletion and stimulating several cellular mechanisms to enhance energy production and to limit energy utilization. AMPK has previously been shown to downregulate the epithelial Na^+ channel ENaC, an effect mediated by the ubiquitin ligase Nedd4-2. The present study explored whether AMPK regulates KCNQ1/KCNE1. To this end, cRNA encoding KCNQ1/KCNE1 was injected into *Xenopus* oocytes with and without additional injection of wild type AMPK (AMPKα1 + AMPKβ1 + AMPKγ1), of the constitutively active $^{\gamma R70Q}$AMPK (α1β1γ1(R70Q)), of the kinase dead mutant $^{\alpha K45R}$AMPK (α1(K45R)β1γ1), or of the ubiquitin ligase Nedd4-2. KCNQ1/KCNE1 activity was determined in two electrode voltage clamp experiments. Moreover, KCNQ1 abundance in the cell membrane was determined by immunostaining and subsequent confocal imaging. As a result, wild type and constitutively active AMPK significantly reduced KCNQ1/KCNE1-mediated currents and reduced KCNQ1 abundance in the cell membrane. Similarly, Nedd4-2 decreased KCNQ1/KCNE1-mediated currents and KCNQ1 protein abundance in the cell membrane. Activation of AMPK in isolated perfused proximal renal tubules by AICAR (10 mM) was followed by significant depolarization. In conclusion, AMPK is a potent regulator of KCNQ1/KCNE1.

Keywords: *AMPK, ischemia, cardiac action potential*

Introduction

The K^+ channel KCNEx/KCNQ1 (KCNE1 was formerly called mink or IsK and KCNQ1 was also named KvLQT1 or Kv7.1) is expressed in a variety of tissues including the heart (Barhanin et al. 1996, Sanguinetti et al. 1996), skeletal muscle (Finsterer and Stollberger 2004) and several epithelia, such as the *stria vascularis* (Wangemann 2006), the renal proximal tubule (Vallon et al. 2001), the gastric parietal cells (Dedek and Waldegger 2001, Grahammer et al. 2001, Heitzmann et al. 2004), intestinal cells (Sugimoto et al. 1990, Schroeder et al. 2000, Dedek and Waldegger 2001, Nicolas et al. 2001, Vallon et al. 2001, Heitzmann et al. 2004) and hepatocytes (Demolombe et al. 2001, Lan et al. 2005, Lan et al. 2006).

Genetic defects of KCNE1 or KCNQ1 lead to Romano Ward syndrome, a disorder characterized by Long QT syndrome and cardiac arrhythmia predisposing to sudden cardiac death (Chiang and Roden 2000). Severe genetic loss-of-function defects in KCNQ1/KCNE1 lead to the Jervell and Lange-Nielson syndrome in humans comprising Long QT syndrome and deafness (Barhanin et al. 1996, Sanguinetti et al. 1996, Neyroud et al. 1997). KCNQ1 polymorphisms have further been associated with diabetes (Unoki et al. 2008, Yasuda et al. 2008).

KCNQ1 knockout mice are deaf and display a shaker/waltzer phenotype (Lee et al. 2000, Casimiro

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et al. 2001), defective gastric acid secretion (Scarff et al. 1999, Lee et al. 2000), vitamin B_{12} deficiency with anemia, blunted stimulation of intestinal Cl^- secretion by cAMP, intestinal loss of Na^+ and K^+, as well as impaired renal and intestinal substrate transport (Vallon et al. 2005). Moreover, KCNQ1 participates in cell volume regulation (Grunnet et al. 2003, Lan et al. 2005, 2006, Bachmann et al. 2007, vanTol et al. 2007).

KCNQ1/KCNE1 activity is decreased and thus action potential duration enhanced by ischemia (Liu et al. 2007). Cellular mechanisms accounting for the downregulation of KCNQ1/KCNE1 activity during ischemia have remained elusive. Candidates include the AMP-activated protein kinase (AMPK), which is activated upon cellular energy depletion. The kinase senses the cytosolic AMP/ATP concentration ratio and thus the energy status of the cell (Towler and Hardie 2007, Winder and Thomson 2007). AMPK stimulates cellular glucose uptake, glycolysis, fatty acid oxidation and enzymes required for ATP production (Ojuka et al. 2000, Winder et al. 2000, Zheng et al. 2001, MacLean et al. 2002, Jessen et al. 2003, Li et al. 2004, Luiken et al. 2004, Lei et al. 2005, Walker et al. 2005, Carling 2007, Jensen et al. 2007, Natsuizaka et al. 2007, Winder and Thomson 2007, Guan et al. 2008, Horie et al. 2008, Park et al. 2009). AMPK thus enhances the cellular ATP generation (McGee and Hargreaves 2008). It further inhibits several energy-utilizing mechanisms, such as protein synthesis, gluconeogenesis and lipogenesis (Carling 2007, Winder and Thomson 2007, McGee and Hargreaves 2008). AMPK stimulates glucose uptake (Carling 2007, Winder and Thomson 2007), an effect largely due to activation of the facilitative glucose carriers GLUT1, GLUT2, GLUT3 and GLUT4 (Ojuka et al. 2000, Winder et al. 2000, Zheng et al. 2001, MacLean et al. 2002, Jessen et al. 2003, Li et al. 2004, Luiken et al. 2004, Lei et al. 2005, Walker et al. 2005, Natsuizaka et al. 2007, Guan et al. 2008, Park et al. 2009) and of the secondary active SGLT1 carrier (Sopjani et al. 2010). Accordingly, AMPK confers some protection against cell death during energy depletion (Hardie 2004, McGee and Hargreaves 2008, Foller et al. 2009).

AMPK has been shown to control the membrane abundance of the epithelial Na^+ channel ENaC, an effect mediated by the ubiquitin ligase Nedd4-2 (Hallows et al. 2003a, Carattino et al. 2005, Bhalla et al. 2006, Almaca et al. 2009).

The present study explored whether AMPK regulates KCNQ1/KCNE1 channels. To this end, voltage-gated current was determined in *Xenopus* oocytes expressing KCNQ1/KCNE1 with or without wild type, constitutively active and inactive AMPK variants. Moreover, the KCNQ1 protein abundance at the cell membrane was determined by immunohistochemistry and confocal microscopy. Additional experiments explored whether the effect of AMPK is mimicked by coexpression of Nedd4-2. Finally, the potential difference across the basolateral membrane in the proximal renal tubule was studied without and upon activation of AMPK.

Methods

Constructs

For generation of cRNA, constructs were used encoding wild type human KCNQ1/KCNE1 (Seebohm et al. 2008, Henrion et al. 2009), wild type AMPKα1-HA, AMPKβ1-Flag, AMPKγ1-HA (Fraser et al. 2007), constitutively active ^{R70Q}AMPKγ1-HA (Hamilton et al. 2001), kinase dead mutant ^{K45R}AMPKα1-HA (Hallows et al. 2003a), wild type AMPK α2-HA (Steinberg and Kemp 2009), wild type Nedd4-2 (Boehmer et al. 2008a) and Nedd4-2^{S795A} lacking an AMPK phosphorylation site [refer to "site directed mutagenesis"]. The AMPK inhibitor compound C (Calbiochem, Bad Soden, Germany) was used at a concentration of 10 μM, dibutyril-cAMP (Sigma, Schnelldorf, Germany) at a concentration of 1 mM.

Voltage clamp in Xenopus oocytes

Xenopus oocytes were prepared as previously described (Boehmer et al. 2008b, Laufer et al. 2009). cRNA encoding KCNQ1 (1.5 ng) and 1.5 ng cRNA encoding KCNE1 were injected with or without 4.6 ng of cRNA encoding either AMPKα1-HA + AMPKβ1-Flag + AMPKγ1-HA (^{WT}AMPK), or AMPKα1-HA + AMPKβ1-Flag + ^{R70Q}AMPKγ1-HA ($^{\gamma R70Q}$AMPK) or ^{K45R}AMPKα1KD-HA + AMPKβ1-Flag + AMPKγ1-HA ($^{\alpha K45R}$AMPK) or AMPKα2-HA + AMPKβ1-Flag + ^{R70Q}AMPKγ1-HA ($^{\gamma R70Q}$AMPKα2) and with or without 5 ng cRNA encoding Nedd4-2 or Nedd4-2^{S795A} on the day of preparation of the *Xenopus* oocytes. All experiments were performed at room temperature 3 or 4 days (Nedd4-2) after injection. In two-electrode voltage-clamp experiments KCNQ1/KCNE1 channel currents were elicited every 20 s with 5 s depolarizing pulses to +80 mV applied from a holding potential of −120 mV. Pulses were applied in 20 mV increments. The data were filtered at 1 kH and recorded with a Digidata 1322A A/D-D/A converter and Chart V.4.2 software for data acquisition and analysis (Axon Instruments) (Ureche et al. 2008).

The analysis of the data was performed with Clampfit 8 (Axon Instruments) software. Activating current traces were fitted using the simplex algorithm to one exponential function: $y = A_0 + A_1 * \exp(-t/\tau)$.

Immunohistochemistry

After 4% paraformaldehyde fixation for at least 12 h, oocytes were cryoprotected in 30% sucrose, frozen in mounting medium and placed on a cryostat (Gehring et al. 2009). Sections were collected at a thickness of 8 µm on coated slides and stored at −20°C. For immunostainings, sections were dehydrated at room temperature, fixated in acetone/methanol (1:1) for 15 min at room temperature, washed in PBS and pre-incubated for 1 h in 5% bovine serum albumin in PBS. The primary antibody used was rabbit anti-KCNQ1 antibody (diluted 1:500, Abcam, Cambridge, UK). Incubation was performed in a moist chamber overnight at 4°C. Binding of primary antibody was visualised with a goat anti-rabbit conjugated FITC antibody (diluted 1:500, Invitrogen, United States). Then, oocytes were analyzed by a fluorescence laser scanning microscope (LSM 510, Carl Zeiss MicroImaging GmbH, Germany) with A-Plan 40x/1.2W DICIII. Brightness and contrast settings were kept constant during imaging of all oocytes in each injection series.

Western blot

For western blotting, 20 intact healthy oocytes were homogenized with a pestle in 400 µl Buffer-H (100mM NaCl, 20mM Tris-HCl, pH 7.4, 1% Triton X-100, and Complete Protease Inhibitor [Roche Diagnostics GmbH, Mannheim, Germany]). The samples were kept at 4°C for 1 h on a rotator, then centrifuged for 2 min at 13,000 rpm. After measurement of the total protein concentration (Bradford assay), 50 µg of protein were solubilized in Roti-Load1 Buffer (Carl Roth GmbH, Karlsruhe, Germany) at 95°C for 10 min and resolved by 10% SDS-PAGE. For immunoblotting proteins were electro-transferred onto a nitrocellulose membrane and blocked with 5% non-fat milk in TBS-0.10% Tween 20 at room temperature for 1 h. The membrane was then incubated with rabbit anti-KCNQ1 antibody (diluted 1:500, Abcam, Cambridge, UK) at 4°C overnight. After washing (TBST), the blot was incubated with secondary anti-rabbit HRP antibody (diluted 1:1000, Cell Signaling Technology, Danvers, MA, USA) for 1 h at room temperature. For loading control the blot was stripped in stripping buffer (Carl Roth GmbH, Karlsruhe, Germany) at 56°C for 30 min. After washing with TBST the blot was blocked with 5% non-fat milk in TBST for 1 h at room temperature. The blot was then incubated with a rabbit anti-GAPDH antibody (diluted 1:1000, Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. After washing with TBST, the blot was incubated with anti-rabbit HRP antibody (diluted 1:1000, Cell Signaling Technology, Danvers, MA, USA) for 1 h at room temperature. Antibody-binding was detected with the ECL detection reagent (Amersham, Freiburg, Germany). Bands were quantified with Quantity One Software (Biorad, München, Germany).

Site-directed mutagenesis

The mutated human Nedd4-2^{S795A} was generated by site-directed mutagenesis (QuikChange II Site-Directed Mutagenesis Kit; Stratagene, Heidelberg, Germany) according to the manufacturer's instructions. The following primers were used: Nedd4-2^{S795A} s: 5′ GGATTTGAAGCCCAATGG GGCAGAAATAATGGTCACAAA 3′ and Nedd4-2^{S795A} as: 5′ TTTGTGACCATTATTTCT GCCCCATTGGGCTTCAAATCC 3′. The mutant was sequenced to verify the presence of the desired mutation.

(Consensus motive for AMPK substrate recognition)

where: Φ is Hydrophobic residue; B is Basic residue; and S^T is Phosphorylated serine residue.

Potential difference across the basolateral cell membrane of isolated perfused proximal straight tubules

The potential difference across the basolateral cell membrane (PDbl) was determined following incubation of isolated renal tubules from C57 BL/6 mice for 1 h at 22°C in the absence or presence of AMPK stimulator AICAR (10 mM). The bath and luminal perfusates were composed of (all numbers mmol/l): 120 NaCl, 5 KCl, 20 $NaHCO_3$, 1.3 $CaCl_2$, 1 $MgCl_2$, 2 Na_2HPO_4. PDbl was measured by a high impedance electrometer (FD223, WPI, Science Trading, Frankfurt, Germany) connected with the electrode via an Ag/AgCl half cell. An Ag/AgCl

reference electrode was connected to the bath. Entry of positive charge by electrogenic transport is expected to depolarize the basolateral cell membrane. The magnitude of the depolarization depends on the magnitude of the induced current on the one hand and on the resistances of cell membranes and shunt on the other.

Statistical analysis

Data are provided as means ± SEM, *n* represents the number of experiments. All oocyte experiments were repeated with at least two batches of oocytes; in all repetitions qualitatively similar data were obtained. Data were tested for significance using ANOVA or *t*-test, as appropriate, and results with $p < 0.05$ were considered statistically significant.

Results

AMPK inhibited voltage-gated outward currents in KCNQ1/KCNE1-expressing Xenopus oocytes

In KCNQ1/KCNE1-expressing, but not in water-injected *Xenopus* oocytes, depolarization triggered a slowly activating current (I_{Ks}) with strong outward

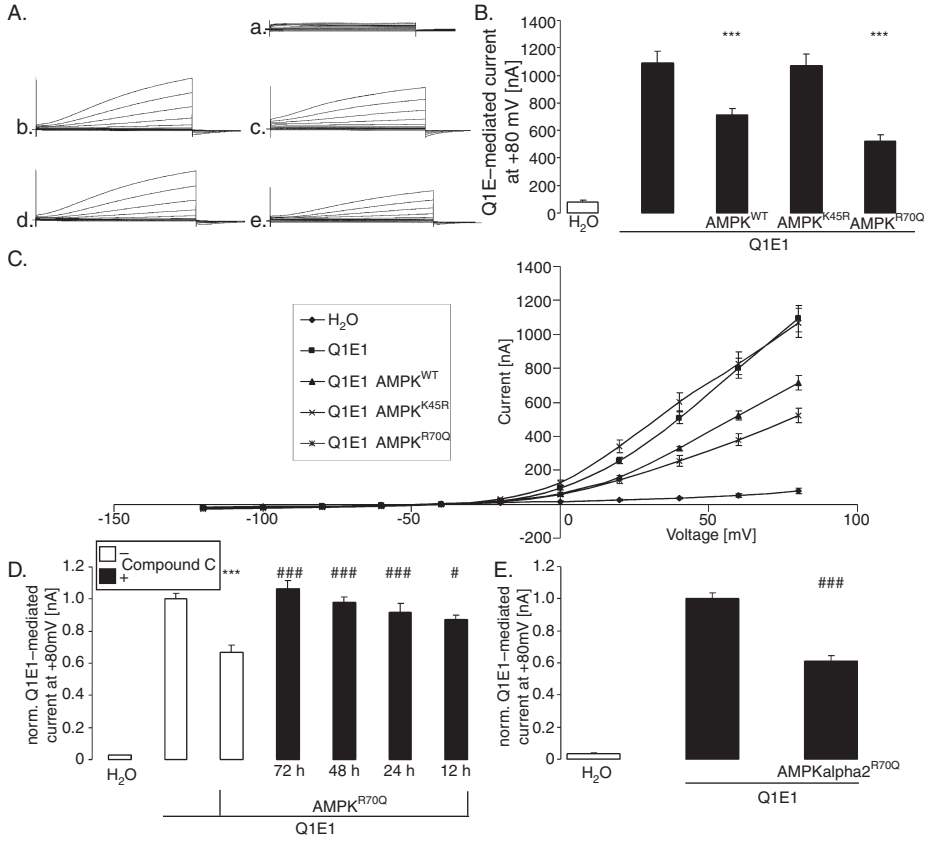

Figure 1. Co-expression of AMPK decreased voltage-gated outward current in KCNQ1/KCNE1 expressing *Xenopus* oocytes. (A) Original tracings of the current induced by depolarization from −60 mV to −40, −20, 0, 20, 40, 60, and 80 mV in *Xenopus* oocytes injected with water (a), expressing KCNQ1/KCNE1 without (b) or with (c) additional co-expression of wild type AMPK, of kinase dead mutant $^{\alpha K45R}$AMPK (d) or of constitutively active $^{\gamma R70Q}$AMPK (e). (B) Arithmetic means ± SEM (n = 22–36) of depolarization-induced K^+ current at +80 mV in *Xenopus* oocytes injected with water (1st bar), expressing KCNQ1/KCNE1 without (2nd bar) or with additional coexpression of wild type AMPK (3rd bar), of kinase dead mutant ^{K45R}AMPK (4th bar) or of constitutively active ^{R70Q}AMPK (5th bar). ***($p < 0.001$) indicates statistically significant difference from the values obtained in oocytes expressing KCNQ1/KCNE1 alone. (C) Arithmetic means ± SEM of depolarization-induced current (I_g) as a function of the potential in *Xenopus* oocytes injected as in B. (D) Arithmetic means ± SEM (n = 12–24) of depolarization-induced K^+ current at +80 mV in *Xenopus* oocytes injected with water (1st bar), expressing KCNQ1/KCNE1 without (2nd bar) or with additional co-expression of constitutively active ^{R70Q}AMPK (3rd bar). The oocytes were incubated in the absence (white bars) or presence of 10 μM AMPK inhibitor compound C (black bars) for the indicated number of hours prior to the experiment. ***($p < 0.001$) indicates statistically significant difference from the values obtained in oocytes expressing KCNQ1/KCNE1 alone. #, ### ($p < 0.05$, $p < 0.001$) indicate significant difference from the absence of compound C. (E) Arithmetic means ± SEM (n = 8–17) of depolarization-induced K^+ current at +80 mV in *Xenopus* oocytes injected with water (1st bar), expressing KCNQ1/KCNE1 without (2nd bar) or with additional coexpression of constitutively active ^{R70Q}AMPKα2 (3rd bar). ### ($p < 0.001$) indicates significant difference from the absence of ^{R70Q}AMPKα2.

rectification (Figure 1A–C). Coexpression of the AMP-activated protein kinase (AMPKα1 + AMPKβ1 + AMPKγ1) was followed by a significant decrease of I_{Ks} by 41 ± 7% (n = 4 batches of 25–36 oocytes) at +80 mV. Furthermore, coexpression of the constitutively active ^{R70Q}AMPK (AMPKα$_1$ + AMPKβ$_1$ + ^{R70Q}AMPKγ$_1$) similarly decreased the slowly activating outward current of KCNQ1/KCNE1. In contrast, coexpression of the inactive ^{K45R}AMPK mutant [^{K45R}AMPKα1 + AMPKβ1 + AMPKγ1] did not significantly modify the slowly activating outward current of KCNQ1/KCNE1-expressing *Xenopus* oocytes. In addition, pharmacological inhibition of AMPK by compound C (10 μM) significantly blocked the AMPK effect on KCNQ1/E1-mediated currents (Figure 1D). Thus, kinase activity is required for the effect of AMPK on KCNQ1/KCNE1 activity.

Another series of experiments tested whether AMPKα2 similarly decreases KCNQ1/KCNE1 activity. As shown in Figure 1E, the constitutively active ^{R70Q}AMPKα2 indeed also decreases KCNQ1/KCNE1-mediated currents.

To test, whether AMPK changes the activation kinetics of the channel the activation constant τ was determined. As shown in Figure 2, coexpression of constitutively active ^{R70Q}AMPK did not significantly modify τ. Further experiments aimed to investigate whether PKA-dependent stimulation of KCNQ1/KCNE1 was modified by AMPK. To this end, KCNQ1/KCNE1-dependent currents were measured in the presence or absence of dibutyril-cAMP (1 mM) for three days. As a result, exposure to dibutyril-cAMP significantly increased the normalized current to 1.29 ± 0.06 rel. units (n = 25 oocytes) whereas

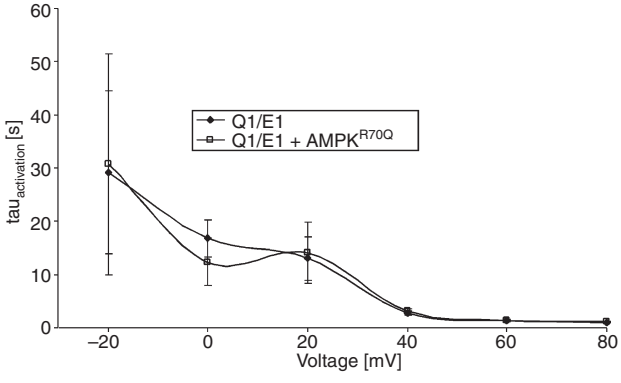

Figure 2. Co-expression of AMPK did not significantly modify activation kinetics of voltage-gated outward currents in KCNQ1/KCNE1-expressing *Xenopus* oocytes. Arithmetic means ± SEM (n = 31–36) of the time constant (τ) plotted vs. the depolarizing potential in *Xenopus* oocytes expressing KCNQ1/KCNE1 alone (closed symbols) or KCNQ1/KCNE1 together with constitutively active ^{R70Q}AMPK (open symbols).

coexpression of constitutively active ^{R70Q}AMPK significantly reduced the normalized current to 0.72 ± 0.03 rel. units (n = 23 oocytes). Most importantly, dibutyril-cAMP failed to significantly modify the normalized KCNQ1/KCNE1-mediated current in oocytes co-expressing constitutively active ^{R70Q}AMPK (0.73 ± 0.04, n = 22 oocytes). Thus, PKA fails to stimulate KCNQ1/KCNE1-dependent currents in oocytes expressing constitutively active ^{R70Q}AMPK.

AMPK decreased the KCNQ1 protein abundance in the cell membrane

A decrease of the slowly activating outward current could have resulted from a decrease of KCNQ1/KCNE1 protein abundance in the cell membrane. To test this possibility, the KCNQ1 protein abundance was determined by confocal microscopy in *Xenopus* oocytes injected with water and in oocytes expressing KCNQ1/KCNE1 alone or together with AMPK. As shown in Figure 3A, the KCNQ1 cell surface expression of the channel protein in *Xenopus* oocytes injected with cRNA encoding KCNQ1/KCNE1 was indeed decreased by the co-expression of wild-type or constitutively active AMPK. The total protein abundance was not affected by AMPK (Figure 3B).

KCNQ1 protein abundance is decreased by the ubiquitin ligase Nedd4-2

To test whether KCNQ1/KCNE1 is regulated by the AMPK-sensitive ubiquitin ligase Nedd4-2, KCNQ1/KCNE1 was expressed together with or without Nedd4-2, with or without constitutively active ^{R70Q}AMPK. As shown in Figure 4A and 4B, Nedd4-2 indeed decreased KCNQ1/KCNE1-dependent currents. In another series of experiment, the effect of Nedd4-2 on KCNQ1/KCNE1-dependent currents was significantly reduced by co-expression of constitutively active ^{R70Q}AMPK (Figure 4C). Nedd4-2^{S795A} which lacks an AMPK phosphorylation site similarly decreased KCNQ1/KCNE1-dependent currents (Figure 4C). Constitutively active ^{R70Q}AMPK, however, failed to significantly modify the Nedd4-2^{S795A} action on KCNQ1/KCNE1-dependent currents (Figure 4C). Additional experiments were performed to determine whether Nedd4-2 is effective through altering KCNQ1 protein abundance in the cell membrane. The KCNQ1 protein abundance in the cell membrane was determined in oocytes expressing KCNQ1/KCNE1 together with or without Nedd4-2, with or without constitutively ^{R70Q}AMPK. As shown in Figure 5, the AMPK effect on KCNQ1 protein cell membrane abundance was indeed mimicked by co-expression of Nedd4-2.

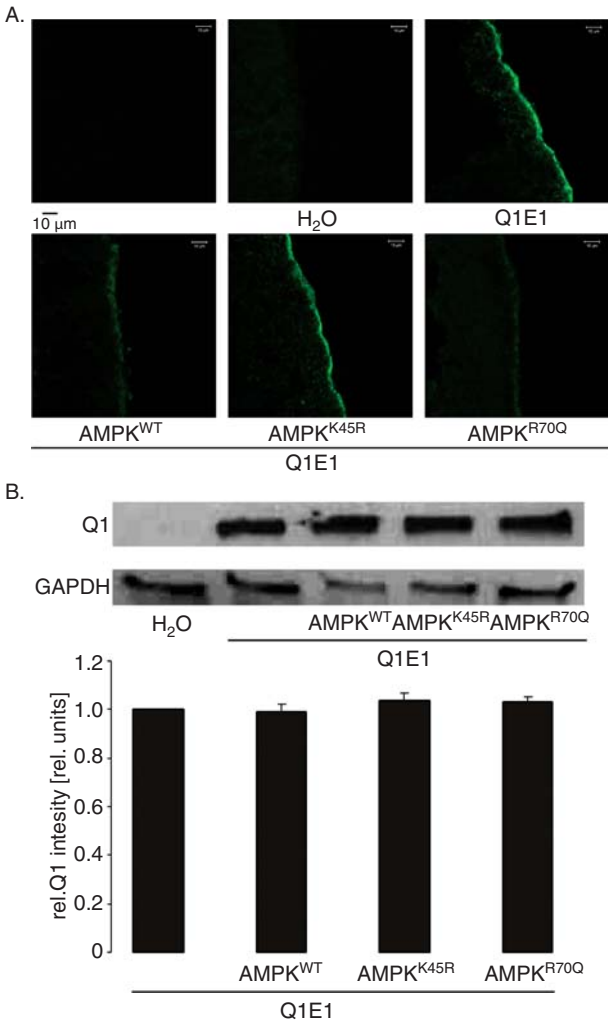

Figure 3. Co-expression of AMPK decreased the KCNQ1 protein abundance within the plasma membrane of oocytes. (A) Confocal images of KCNQ1 protein abundance in the plasma membrane of *Xenopus* oocytes injected with water (2nd upper panel), expressing KCNQ1/KCNE1 without (3rd upper panel) or with additional co-expression of wild type AMPK (1st lower panel), of kinase dead mutant $^{\alpha K45R}$AMPK (2nd lower panel) or of constitutively-active $^{\gamma R70Q}$AMPK (3rd lower panel). The cells were subjected to immunofluorescent staining using FITC-conjugated antibody (grey/green). The 1st upper panel serves as control (absence of primary antibody). (B) Original Western Blots of total KCNQ1 (upper panel) and GAPDH (lower panel) in *Xenopus* oocytes injected with water (1st lane), expressing KCNQ1/KCNE1 without (2nd lane) or with additional co-expression of wild-type AMPK (3rd lane), of kinase dead mutant $^{\alpha K45R}$AMPK (4th lane) or of constitutively-active $^{\gamma R70Q}$AMPK (5th lane). The lower bar diagram displays the densitometric analysis of the Western blots (arithmetic means ± SEM [n = 3]).

Stimulation of AMPK depolarizes proximal renal tubule cells

Isolated proximal renal tubules from C57 BL/6 mice express both, AMPK and KCNQ1 (Figure 6A). To test whether AMPK activity depolarizes proximal tubular cells as suggested by AMPK-dependent inhibition of KCNQ1/KCNE1, the potential difference across the basolateral cell membrane (PD bl) of isolated perfused proximal tubular cells was determined following incubation in the presence and absence of the AMPK stimulator AICAR (10 mM) for 1 h. As shown in Figure 6B, AICAR indeed depolarized proximal tubular cells.

Discussion

The present study reveals a novel regulator of the slowly activating outward current generated by the heterotetrameric K^+ channel KCNQ1/KCNE1. The AMP-activated protein kinase AMPK downregulates the channel and thus decreases K^+ conductance and repolarization.

The AMPK-dependent downregulation of KCNQ1 is at least partially due to stimulation of the ubiquitin ligase Nedd4-2. AMPK has previously been shown to phosphorylate Nedd4-2 (Bhalla et al. 2006) thus influencing the interaction of the ubiquitin ligase with the epithelial Na^+ channel ENaC (Carattino et al. 2005, Bhalla et al. 2006, Almaca et al. 2009).

According to the present observations, AMPK further disrupts the well known (Boucherot et al. 2001, Marx et al. 2002, Dilly et al. 2004, Nicolas et al. 2008, Dai et al. 2009) stimulation of KCNQ1 by cAMP. The AMPK-dependent regulation of the Cl^- channel CFTR (Hallows et al. 2000, 2003a, 2003b, 2006, 2010, Crawford et al. 2006, Muimo et al. 2006, Mehta 2007) involves the phosphorylation of the R domain of the channel thus decreasing the activation of CFTR by protein kinase A (Walker et al. 2003, King et al. 2009, Kongsuphol et al. 2009a, 2009b).

In contrast to KCNQ1/KCNE1 activity, AMPK stimulates the activity of the facilitative glucose carriers GLUT1, GLUT2, GLUT3 and GLUT4 thus increasing cellular glucose uptake (Ojuka et al. 2000, Winder et al. 2000, Zheng et al. 2001, MacLean et al. 2002, Jessen et al. 2003, Li et al. 2004, Luiken et al. 2004, Lei et al. 2005, Walker et al. 2005, Natsuizaka et al. 2007, Guan et al. 2008, Park et al. 2009). The glucose uptake serves to provide the cell with fuel. Beyond that AMPK stimulates glycolysis, fatty acid oxidation and expression of enzymes required for ATP production (Carling 2007, Winder and Thomson 2007). All those functions counteract ATP depletion.

Inhibition of KCNQ1 by AMPK may be considered a double-edged sword. On the one hand, inhibition of K^+ channels depolarizes the cell membrane, fostering Cl^- entry and potentially deleterious cell

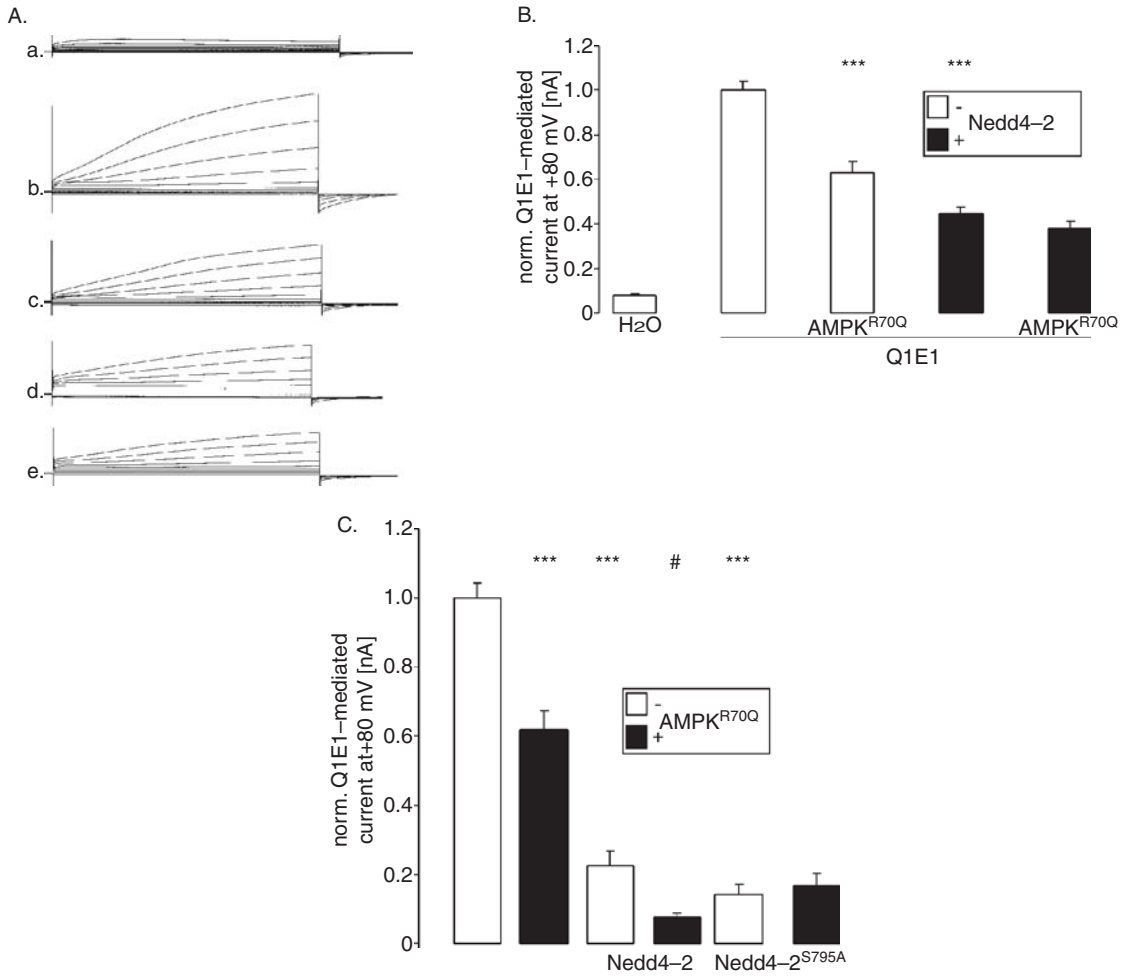

Figure 4. Similar to $^{\gamma R70Q}$AMPK the ubiquitin ligase Nedd4-2 downregulated KCNQ1/KCNE1. (A) Original tracings of the current induced by depolarization from −60 mV to −40, −20, 0, 20, 40, 60, and 80 mV in *Xenopus* oocytes injected with water (a), expressing KCNQ1/KCNE1 without (b,d) or with (c,e) additional coexpression of constitutively active $^{\gamma R70Q}$AMPK in the absence (b,c) or presence (d,e) of Nedd4-2. (B) Arithmetic means ± SEM (n = 10–19) of depolarization-induced current at +80 mV in *Xenopus* oocytes injected with water (H_2O), expressing KCNQ1/KCNE1 (Q1E1) without or with additional co-expression of constitutively active ^{R70Q}AMPK ($AMPK^{R70Q}$) in the absence (open bars) or presence (closed bars) of Nedd4-2. *** ($p < 0.001$) indicates statistically significant difference from the values obtained in oocytes expressing KCNQ1/KCNE1 alone. (C) Arithmetic means ± SEM (n = 11–14) of depolarization-induced current at +80 mV in *Xenopus* oocytes injected with KCNQ1/KCNE1 without (white bars) or with additional coexpression of constitutively active ^{R70Q}AMPK (black bars) in the absence (2 left bars) or presence of Nedd4-2 (2 middle bars) or of Nedd4-2^{S795A} (2 right bars). *** ($p < 0.001$) indicates statistically significant difference from the values obtained in oocytes expressing KCNQ1/KCNE1 alone. # ($p < 0.05$) indicates statistically significant difference from the absence of ^{R70Q}AMPK.

swelling (Lang et al. 1986, 1998). In the heart, inhibition of KCNQ1 is expected to delay repolarization thus jeopardizing cardiac function (Peroz et al. 2008). On the other hand, inhibition of K^+ channels could decrease energy expenditure. In the proximal renal tubule, for instance, inhibition of K^+ channels decreases the driving force for Na^+-coupled transport of glucose and other substrates across the apical membrane and at the same time decreases electrogenic HCO_3^- exit across the basolateral cell membrane leading to cytosolic alkalinization and subsequent inhibition of the apical Na^+/H^+ exchanger (Lang and Rehwald 1992). Thus, depolarization curtails Na^+ entry and thus decreases the requirement for energy-consuming Na^+ extrusion by the Na^+/K^+ ATPase (Lang and Rehwald 1992). Inhibition of KCNQ1/KCNE1 may further limit the cellular K^+ loss during impaired function of Na^+/K^+ ATPase in energy-depleted cells. Cellular K^+ loss may foster suicidal cell death (Bortner and Cidlowski 2004, Foller et al. 2006, Shimizu et al. 2006, Becker et al. 2007, Schneider et al. 2007). By counteracting HCO_3^- exit depolarization may prevent cytosolic acidification, which accelerates the death of apoptotic cells (Lupescu et al. 2009) and compromises glycolysis (Boiteux and Hess 1981).

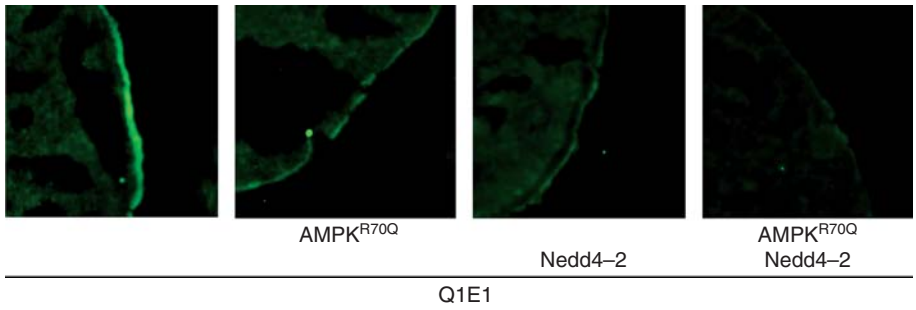

Figure 5. Similar to $^{\gamma R70Q}$AMPK the ubiquitin ligase Nedd4-2 decreased the KCNQ1 protein abundance in the cell membrane. Confocal images of KCNQ1 protein abundance in the plasma membrane of *Xenopus* oocytes expressing KCNQ1/KCNE1 without (1st panel) or with additional coexpression of constitutively active $^{\gamma R70Q}$AMPK (2nd panel), of the ubiquitin ligase Nedd4-2 (3rd panel) or of both, $^{\gamma R70Q}$AMPK and Nedd4-2 (4th panel). The cells were subjected to immunofluorescent staining using FITC-conjugated antibody (grey/green).

The present observations may not only be relevant for ischemia and energy depletion. AMPK is further stimulated by an increase in the cytosolic Ca^{2+} activity (Towler and Hardie 2007), by a decrease of O_2 levels (Evans et al. 2005) and by exposure to nitric oxide (Lira et al. 2007).

Moreover, the AMPK-dependent regulation of KCNQ1 is not only important for cardiac repolarization and maintenance of cell membrane potential in proximal renal tubules of the kidney. In addition to the heart (Barhanin et al. 1996, Sanguinetti et al. 1996, Neyroud et al. 1997) and kidney (Vallon et al. 2001) KCNQ1 is expressed in the liver (Demolombe et al. 2001, Lan et al. 2005, 2006), skeletal muscle (Finsterer and Stollberger 2004) and several epithelia (Sugimoto et al. 1990, Schroeder et al. 2000, Dedek and Waldegger 2001, Grahammer et al. 2001, Nicolas et al. 2001, Vallon et al. 2001, 2005, Heitzmann et al. 2004). In the liver, for instance, KCNQ1 governs cell volume and thus cell volume-sensitive functions including glucose uptake (Boini et al. 2009). Beyond that KCNQ1 is important for a variety of functions including hearing (Lee et al. 2000, Casimiro et al. 2001), gastric acid secretion (Scarff et al. 1999, Lee et al. 2000), as well as intestinal and renal transport (Vallon et al. 2005). AMPK-dependent regulation of KCNQ1 could thus participate in the pleotropic functional consequences of energy depletion in those tissues.

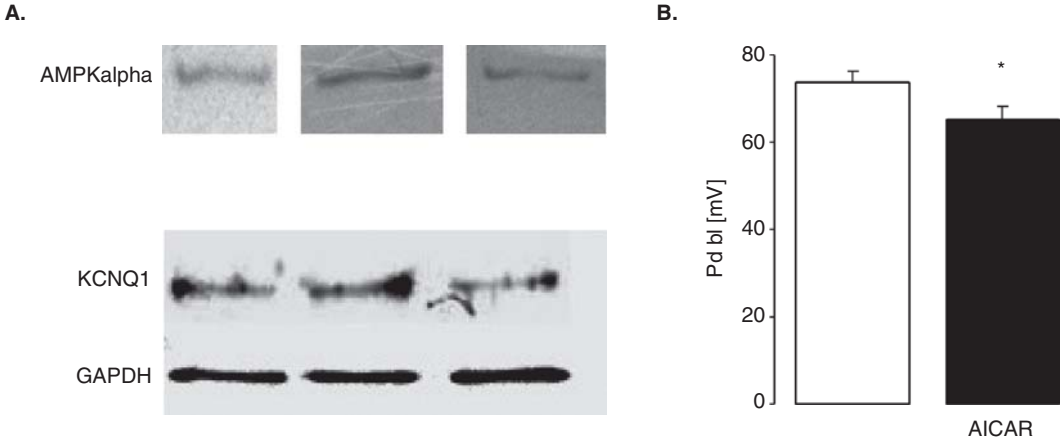

Figure 6. Depolarization of proximal renal tubule cells by stimulation of AMPK (A) Original Western Blots demonstrating expression of AMPKα (upper panel) and KCNQ1 (lower panel; GAPDH was used as loading control) in isolated proximal tubules of C57 BL/6 mice. (B) Arithmetic means ± SEM ($n = 7–8$) of the potential difference across the basolateral membrane (PD) of isolated perfused proximal renal tubules from C57 BL/6 mice incubated for 1 h at 22°C in the absence (left bar) or presence (right bar) of AMPK stimulator AICAR (10 mM). * ($p < 0.05$) indicates statistically significant difference from the values obtained in absence of AICAR.

Conclusion

The present observations unravel a powerful inhibitory effect of the AMP-activated kinase AMPK on the slowly activating K^+ channels KCNQ1/KCNE1. The effect is likely to profoundly affect cellular functions during energy depletion, hypoxia, excessive cytosolic Ca^{2+} activity, and exposure to nitric oxide.

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