**Intact colonic KCa1.1 channel activity in KCNMB2 knockout mice**

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**Abstract**

Mammalian potassium homeostasis results from tightly regulated renal and colonic excretion, which balances the unregulated dietary K⁺ intake. Colonic K⁺ secretion follows the pump-leak model, in which the large conductance Ca²⁺-activated K⁺ channel (KCa1.1) is well established as the sole, but highly regulated apical K⁺ conductance. The physiological importance of auxiliary β and γ subunits of the pore-forming α-subunit of the KCa1.1 channel is not yet fully established. This study investigates colonic K⁺ secretion in a global knockout mouse of the KCa1.1-β2-subunit (KCNMB2⁻/⁻), which apparently is the only β-subunit of the colonic enterocyte KCa1.1 channel. We can report that: (1) Neither KCa1.1 α- nor the remaining β-subunits were compensatory transcriptional regulated in colonic epithelia of KCNMB2⁻/⁻/⁻ mice. (2) Colonic epithelia from KCNMB2⁻/⁻/⁻ mice displayed equal basal and ATP-induced KCa1.1-mediated K⁺ conductance as compared to KCNMB2⁺/+ mice. (3) K⁺ secretion was increased in KCNMB2⁻/⁻ epithelia compared to wild-type epithelia from animals fed an aldosterone-inducing diet. (4) Importantly, the apical K⁺ conductance was abolished by the specific blocker of KCa1.1 channel iberiotoxin in both KCNMB2⁺/+ and KCNMB2⁻/⁻ mice. Recently a novel family of auxiliary γ-subunits of the KCa1.1 channel has been described. (5) We detected the γ1-subunit (LRRC26) mRNA in colonic epithelia. To investigate the physiological role of the γ1-subunit of KCa1.1 channels in colonic K⁺ secretion, we acquired an LRRC26 knockout mouse. (6) Unexpectedly, LRRC26 mice had a perinatal lethal phenotype, thus preventing functional measurements. On this basis we conclude that colonic K⁺ secretion is intact or even increased in mice lacking the β2-subunit of KCa1.1 channel complex despite no additional compensatory induction of KCa1.1 β-subunits.

**Introduction**

Whole body K⁺ homeostasis is achieved by matching renal and colonic K⁺ excretion to dietary K⁺ intake. In the murine distal colon, K⁺ secretion takes place in the colonic crypts (Sorensen et al. 2011). According to the pump-leak model, K⁺ enters the crypt cells via the basolateral Na⁺/K⁺-ATPase or the Na⁺/K⁺/2Cl⁻-cotransporter (NKCC1) and exits over the luminal membrane via the KCa1.1 channel complexes (Sausbier et al. 2006). The KCa1.1 channel consists of pore-forming α-subunits and regulatory β-subunits, of which there are four different isoforms (β1-4) (Orio et al. 2002; Lippiat et al. 2003). The different β-subunit isoforms confer different activation and inactivation properties to the KCa1.1 channel, but all increases the Ca²⁺ sensitivity of the channel, thus facilitating channel activity (Brenner et al. 2000a; Lippiat et al. 2003; Orio and Latorre 2005). The α-subunit of the KCa1.1 channels is ubiquitously expressed. Tissue specific coexpression of the different β-subunits is an important way, in which KCa1.1 channel function can be tailored to cell type specific functions. In the murine distal colonic...
epithelium, KCa1.1 channels are composed exclusively of α- and β2-subunits (Sorensen et al. 2008).

The β2-subunit is unique among the KCa1.1 β-subunits as it acts in both activating and inactivating properties to the KCa1.1 channel (Lippiat et al. 2003; Oriol and Latorre 2005). Coexpression of α- and β2-subunits increase open probability of the channel by increasing Ca2+-sensitivity of the channel, when compared to channels composed of α-subunits only (Lippiat et al. 2003; Oriol and Latorre 2005). On the other hand, β2-subunits mediate rapid inactivation of the KCa1.1 channel via an N-terminal ball-and-chain domain on the β2-subunit (Bentrop et al. 2001) and decrease surface expression of the α-subunit. The latter is suggested to be mediated by an endocytic sorting signal in the β2-subunit (Zarei et al. 2007). Because of the dual nature of β2-mediated regulation of the KCa1.1 channel, it is difficult to predict the overall effect of removal of β2-regulation of KCa1.1 channels in the murine distal colon.

In this study, we use a mouse model with a global deletion of the β2-subunit (KCNMB2 knockout) of the KCa1.1 channel to investigate the functional role of the β2-subunit in distal colonic K+ secretion. In accordance with our previous work, we found that murine distal colonic epithelial cells express α- and β2-subunits (Sorensen et al. 2008). KCa1.1 channels that are composed of α-subunits only require substantial membrane depolarization to open (Oriol et al. 2002; Lippiat et al. 2003). This raises the question as to how distal colonic KCa1.1 channel activity is supported in KCNMB2−/− mice. One answer could be provided by the family of γ-subunits (γ1-4, encoded by the genes LRRC26, LRRC52, LRRC55, and LRRC38, respectively) recently described for the KCa1.1 channel. These γ-subunits similar to the β-subunits modulate KCa1.1 channel function (Yan and Aldrich 2010, 2012). LRRC26 mRNA expression has been reported in whole human colon (Yan and Aldrich 2012), and we demonstrate mRNA expression of LRRC26 in the distal colonic epithelium. To address whether γ1-subunits support KCa1.1 channel activity in the murine distal colon, we acquired a global LRRC26 knockout mouse. LRRC26−/− mice did not survive after birth, precluding a description of distal colonic K+ secretion in these mice.

Materials and Methods

Mice, diets, and housing

The KCNMB2 strain was generated in the 129/SvEvBrd x C57BL/6 background by the Texas Institute of Genomic Medicine (College Station, TX). The KCNMB2 gene was disrupted by truncation of exon 3 and deletion of exons 4 and 5. Mice were bred from heterozygous families, yielding KCNMB2+/+ and KCNMB2−/− littersmates. Mice were genotyped as described previously (Larsen et al. 2016). The LRRC26 strain was generated in the C57BL/6NTac background by KOMP, Davis, CA. The LRRC26 gene was disrupted by insertion of a cassette encoding the LacZ and neomycin resistance genes. Mice were bred from heterozygous breeding families. Genotyping was performed with the primers WT-Fw: 5’-ATATTGGAATCGCCCTTCTAAATCCC-3’, WT-Rev: 5’-GTTCTCCCCTCAGATCTTAGTATAGCAGC-3’, KO-Fw: 5’-GAGCCCTGTGTTCCACATACTTCA-3’, and KO-Rev: 5’-TAGCATGACTAGCTCTCCCCAGTG-3’. To determine whether LRRC26 ablation resulted in early termination of embryos, late gestational fetuses were isolated from two pregnant females.

The mice were given ad libitum access to food and water, and kept in cages with not more than four mice per cage. Mice were fed either a normal diet (Altromin 1310, 10 g K+/kg, 2 g Na+/kg) or an aldosterone-inducing high K+/low Na+ diet (Altromin 1350, 50 g K+/kg, 0.2 g Na+/kg). Both male and female mice were used at the age between 6 and 16 weeks. All experiments were performed in accordance with the Danish legislation on the protection of animals.

Isolation of distal colonic epithelium

The most distal 4 cm of the colon was dissected free and feces content was flushed out with ice-cold Ca2+-free Ringer. Colon was inverted by inserting a glass rod into the colon via the anus, tying a suture around colon and glass rod at the proximal end and retracting the glass rod. The distal 2 cm of the inverted colon was tied at both ends with suture and the sack was filled with Ca2+-free Ringer and incubated in Ca2+-free Ringer at 37°C for 10 min while being slowly shaken. After incubation, the inverted colonic sacks were vigorously shaken by hand, causing the epithelium to detach from the colon. The colonic sacks were discarded, and the isolated colonic epithelia in the liquid phase were centrifuged at 10,392g, 4°C for 10 min, yielding pellets of epithelial cells. This preparation has previously been shown to be devoid of smooth muscle contamination (Sorensen et al. 2011).

Semi-quantitative RT-PCR

RNA was isolated from distal colonic epithelia by RNEasy mini kits (Qiagen). Generation of cDNA was performed with Superscript III and Superase (Invitrogen), and semi-quantitative RT-PCR analysis was performed with Taqman Gene Expression Assays (Applied Biosystems) for KCNMA1 (Mm00516078_m1), KCNMB1 (Mm00466621_m1), KCNMB2 (Mm00511481_m1),
KCNMB3 (Mm01292437_m1), KCNMB4 (Mm00465684_m1), LRR2C26 (Mm00525100_g1), LRR2C38 (Mm01718430_m1), LRR2C52 (Mm04204090_m1), LRR2C55 (Mm02600578_m1), HPRT (Mm01545399_m1), and β-actin (Mm00607939_s1). Relative mRNA expression was calculated using the 2^−ΔΔCt-method (Schmittgen and Livak 2008), the average Ct value for HPRT and β-actin was used as reference Ct (Schmittgen and Livak 2008). All expression assays have been verified on positive expressing tissues in a previous publication from our group (Larsen et al. 2016).

Ussing chambers

The most distal 4 cm of the colon was dissected free and feces were flushed out with ice-cold Ringer solution. The most distal 2 cm of the colon was mounted in Ussing chambers with an aperture area of 0.238 cm². The apical and basolateral compartments were perfused with 7.5 mL Ringer solution, bubbled with 5% CO₂, 21% O₂, 74% N₂ and heated to 37°C by a heated water jacket. Experiments were performed in open-circuit mode; transepithelial voltage and resistance was calculated from the transepithelial voltage and resistance. Upon mounting of the tissue in the Ussing chambers, 5 μmol/L of indomethacin was added to both the apical and basolateral compartments and 1 μmol/L tetrodotoxin was added to the basolateral compartment to minimize prostaglandin-mediated and neuronal stimulation of Cl⁻ secretion in response to the mechanical stimulation during the mounting procedure. After mounting, the tissue was allowed to stabilize for 10 min, whereupon 100 μmol/L of amiloride was added to the apical compartment to inhibit ENaC-mediated Na⁺ transport. Five minutes after amiloride application, KCa1.1 channel activity was quantified as the change in Isc induced by luminal application of 5 mmol/L Ba²⁺ (added as BaCl₂). Ba²⁺ is an unspecific K⁺ channel blocker, but can be used to quantify KCa1.1 channel activity in the distal colon as the KCa1.1 channel is the only apical K⁺ channel in this tissue (Sausbier et al. 2006). Experiments with iberiotoxin (IBTX) (Latoxan, Valence, France) were performed to ascertain that the Ba²⁺-sensitive Isc in distal colonic epithelium from KCNMB2-/- mice was in fact mediated by the KCa1.1 channel. Due to the size of IBTX (4230 Da) and the localization of KCa1.1 channels to the apical membrane of mucus covered distal colonic crypts (Soren sen et al. 2011), IBTX diffusion to the site of the KCa1.1 channels is slow. Consequently, the effect of IBTX develops over a longer time (Sorensen et al. 2008, 2010), as opposed to the effects of amiloride and Ba²⁺, which occurs within 30 sec after application (Sausbier et al. 2006; Matos et al. 2007). We quantified the effect of 120 mmol/L luminal IBTX as the Ba²⁺ sensitive short circuit current 30 min after application of IBTX. KCa1.1 channel activity can also be transiently stimulated by application of nucleotides to the apical compartment (Matos et al. 2005). ATP-induced KCa1.1 activation was studied by application of 100 μmol/L ATP to the apical compartment. The maximal (peak) value of the transient changes in short circuit current was quantified.

Solutions

Ussing chamber Ringer: 120 mmol/L NaCl, 0.4 mmol/L KH₂PO₄, 1.6 mmol/L K₂HPO₄, 1 mmol/L MgCl₂, 5 mmol/L glucose, 1.3 mmol/L Ca-glucuronate, 2 mmol/L Na-HEPES. pH was adjusted to 7.4, whereupon 25 mmol/L NaHCO₃ was added to yield a pH of 7.4 when the solution was bubbled with the 5% CO₂, 21% O₂, 74% N₂ gas mixture. Ca²⁺-free Ringer: 127 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L MgCl₂, 5 mmol/L Na-pyruvate, 10 mmol/L Na-HEPES, 5 mmol/L Na-EDTA (C₁₀H₁₆N₂O₈), 5 mmol/L glucose.

Statistics

The assumption of normal distribution of data was tested by the Kolmogorov–Smirnov test. Data were analyzed by Student’s t-test for comparison of two experimental groups or by one-way ANOVA for multiple comparisons. Statistical analysis was performed with the GraphPad Prism software (version 4.02). The Mendelian genotype distributions were tested by a chi-square test for Mendelian ratios by the use of the web page http://www.ihh.kvl.dk/htm/kc/popgen/genetik/applets/ki.htm. The calculated chi-square values were evaluated by the use of a table of percentage point of chi-square distribution.

Results

Expression of KCa1.1 α- and β-subunits in the distal colonic epithelium

We used semi-quantitative rt-PCR to study expression of α- and β-subunits of the KCa1.1 channel in preparations of distal colonic epithelium. We confirmed our previous observation (Sorensen et al. 2008) that only the β2 subunit is expressed at a significant level in the tissue (Fig. 1). β2 mRNA was undetectable in preparations from
KCNMB2<sup>−/−</sup> mice. No compensatory upregulation was observed in either the α-subunit or in any of the remaining β-subunits, although mRNA expression of β1, β3, and β4 was too low (C<sub>T</sub> > 38) to allow quantitative comparison of expression between genotypes.

**Increased aldosterone stimulated colonic K<sub>Ca1.1</sub> activity in KCNMB2<sup>−/−</sup> mice**

The effect of KCNMB2 ablation on distal colonic K<sub>Ca1.1</sub> channel activity was investigated in Ussing chambers. Basal K<sub>Ca1.1</sub> channel activity was quantified as Ba<sup>2+</sup>-sensitive I<sub>sc</sub> and measured to be 9.2 ± 1.0 µA cm<sup>−2</sup> (n = 13) in KCNMB2<sup>+/+</sup> preparations and 12.2 ± 1.1 µA cm<sup>−2</sup> (n = 13) in KCNMB2<sup>−/−</sup> preparations (Fig. 2A, B and E). Colonic K<sub>Ca1.1</sub> channel activity is per se low in rodents kept on a normal diet, because of the low plasma aldosterone levels in animals on this diet. We speculated that differences in K<sub>Ca1.1</sub> channel activity between KCNMB2<sup>+/+</sup> and KCNMB2<sup>−/−</sup> mice might be more visible if the K<sup>+</sup> secretion was stimulated by an aldosterone-inducing diet for 4 days. This maneuver increased plasma aldosterone and distal colonic ENaC activity, the latter measured asamiloride sensitive I<sub>sc</sub> (data presented in table 1). Interestingly, the K<sub>Ca1.1</sub> channel activity was markedly increased in distal colonic epithelia from KCNMB2<sup>−/−</sup> mice, compared to KCNMB2<sup>+/+</sup> mice, when fed an aldosterone-inducing high K<sup>+</sup>/low Na<sup>+</sup> diet for 4 days. The Ba<sup>2+</sup>-sensitive I<sub>sc</sub> was higher in the KCNMB2<sup>−/−</sup> preparations (28.3 ± 2.4 µA cm<sup>−2</sup> [n = 13]) when compared with the KCNMB2<sup>+/+</sup> preparations (20.8 ± 2.1 µA cm<sup>−2</sup> [n = 13]) from animals kept for 4 days on the aldosterone-inducing high K<sup>+</sup>/low Na<sup>+</sup> diet (Fig. 2C, D and E). In summary, Ba<sup>2+</sup>-sensitive baseline colonic K<sub>Ca1.1</sub> activity is not altered by deletion of KCNMB2 under normal dietary conditions.
but is augmented in mice subjected to simultaneous dietary K\(^+\) loading and Na\(^+\) depletion.

**Ba\(^{2+}\)-sensitive short circuit current is inhibited by specific KCa1.1 channel blocker**

To verify whether the Ba\(^{2+}\)-sensitive \(I_{sc}\) in KCNMB2\(^{-/-}\) mice was in fact mediated by the KCa1.1 channel, we performed Ussing chamber experiments, in which 120 mmol/L of the specific KCa1.1 channel inhibitor IBTX was added to the luminal compartment. Thirty minutes after addition of IBTX or vehicle, BaCl\(_2\) (5 mmol/L) was added to the luminal perfusate to assess any Ba\(^{2+}\)-sensitive short circuit was measurable in the presence of IBTX. These experiments were performed with preparations from mice kept on the aldosterone-inducing diet for 4 days. We measured a Ba\(^{2+}\)-sensitive \(I_{sc}\) of 13.2 ± 2.0 \(\mu\)A cm\(^{-2}\) \((n = 6)\) in the KCNMB2\(^{+/+}\) vehicle time controls, whereas Ba\(^{2+}\)-sensitive \(I_{sc}\) was reduced to 3.7 ± 2.3 \(\mu\)A cm\(^{-2}\) \((n = 5)\) in preparations pretreated with IBTX (Fig. 3). The same pattern was observed in KCNMB2\(^{-/-}\) preparations, here we measured 13.9 ± 3.2 \(\mu\)A cm\(^{-2}\) \((n = 6)\) in the vehicle time controls and 0.7 ± 2.6 \(\mu\)A cm\(^{-2}\) \((n = 6)\) in preparations pretreated with IBTX (Fig. 3). These data indicate that no additional K\(^+\) conductance other than the KCa1.1 channel contributes to Ba\(^{2+}\)-sensitive \(I_{sc}\) in KCNMB2\(^{-/-}\) mice.

**ATP-induced KCa1.1 activity was higher in colon from KCNMB2 \(^{-/-}\) mice at high plasma aldosterone**

KCa1.1 channel activity is stimulated by increased intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)). The nucleotides ATP and UTP are known to cause reliable, transient increase in [Ca\(^{2+}\)]\(_i\) in colonic epithelia via luminal P2Y receptors (Matos et al. 2005). Addition of ATP (100 \(\mu\)mol/L) to the luminal perfusion of the Ussing chamber was followed by a transient lumen-positive deflection in transepithelial voltage. This deflection has previously been shown to be caused by opening of apical KCa1.1 channels (Sausbier et al. 2006). The ATP-induced change in \(I_{sc}\) was not different in preparations from the two genotypes (49.3 ± 9.4 \(\mu\)A cm\(^{-2}\) \((n = 6)\) in the KCNMB2\(^{+/+}\) and 31.2 ± 4.8 \(\mu\)A cm\(^{-2}\) \((n = 6)\) in the KCNMB2\(^{-/-}\) preparations) (Fig. 4A, B and E). In tissues from mice kept on the aldosterone-inducing diet for 4 days, a significantly larger peak in ATP-induced \(I_{sc}\) was observed in the KCNMB2\(^{-/-}\) preparations than in KCNMB2\(^{+/+}\) preparations (33.4 ± 3.7 \(\mu\)A cm\(^{-2}\) \([n = 8]\) and 15.6 ± 4.3 \(\mu\)A cm\(^{-2}\) \([n = 8]\) respectively, Fig. 4C, D and E).

**Expression of KCa1.1 \(\gamma\)-subunits in the distal colonic epithelium**

A new family of KCa1.1 channel \(\gamma\)-subunits, which modulate KCa1.1 channel activity similarly to the \(\beta\)-subunits, has recently been described, and mRNA expression of some of these \(\gamma\)-subunits has been detected in whole human colon (Yan and Aldrich 2012). It is possible that the intact KCa1.1 channel activity in KCNMB2\(^{-/-}\) mice could be attributed to expression of one or more of these \(\gamma\)-subunits. We therefore performed semi-quantitative RT-PCR to determine whether any of the \(\gamma\)-subunits were expressed in the murine distal colonic epithelium. We detected high mRNA expression of \(\gamma1\) (LRRC26), whereas mRNA expression of \(\gamma2\), \(\gamma3\) and \(\gamma4\) was absent or very low in colonic epithelium from KCNMB2\(^{+/+}\) mice (Fig. 5). There were no differences in distal colonic mRNA expression in any of the \(\gamma\)-subunits between KCNMB2\(^{+/+}\) and KCNMB2\(^{-/-}\) mice (Fig. 5).

**Post-natal lethality of LRRC26 mice**

To investigate whether the \(\gamma1\)-subunit supports KCa1.1 channel activity in the distal colon, we acquired a knock-out mouse for the \(\gamma1\)-subunit (LRRC26\(^{-/-}\)). In total, we genotyped 77 pups from 10 litters at the time of weaning
(day 21) and this resulted in 24 LRRC26+/+, 53 LRRC26+/- and no LRRC26-/- mice (Fig. 6A). These numbers fit significantly to a normal Mendelian distribution where the LRRC26-/- mice were absent. The breeding families were checked for new offspring every morning, meaning that newborn pups were discovered before they were 24 h old. We did not observe any reduction in the number of pups in the litters between the first observation of pups and the day of weaning. To determine whether LRRC26 ablation resulted from early abortion of LRRC26-/- embryos, we genotyped 20 late gestational fetuses (~day 18) from heterozygous breeding families. No apparent developmental defects were noticeable in any of the 20 fetuses, and the genotype distribution of the 20 fetuses...
were 5 LRRC26+/+, 8 LRRC26+/-, and 7 LRRC26−/− (Fig. 6A). These data indicate that LRRC26 genotypes, as expected, show a Mendelian distribution, but with a lethal phenotype of LRRC26−/−, which manifests during birth or shortly thereafter.

**Discussion**

In this study, we describe the effect of KCNMB2 ablation on distal colonic KCa1.1 channel activity. We confirm our observation, published in 2008, that the β2-subunit of the KCa1.1 channel is the only KCa1.1 β-subunit transcribed in the murine distal colon (Sorensen et al. 2008). Despite the lack of the only apparent β-subunit in the distal colonic epithelium, preparations of the distal colon from KCNMB2−/− mice displayed basal Ba2+-sensitive ISc, which was equal in size to that in KCNMB2+/+ preparations. In preparations from mice kept on an aldosterone-inducing diet, the Ba2+-sensitive ISc was increased in both genotypes, and this to even higher values in the KCNMB2−/− tissues than in KCNMB2+/+ tissues.

On the physiological level, KCa1.1-mediated colonic K+ secretion is tightly regulated. A number of different receptor agonists act as physiologically active secretagogues (Rechkemmer et al. 1996; Matos et al. 2005; Sorensen et al. 2010; Zhang et al. 2012). Luminal ATP has been shown to acutely stimulate colonic K+ secretion mediated via KCa1.1 channels. The physiological purpose of this effect is not clear. It has been suggested that the increased ion and water secretion observed when gastrointestinal tract is stimulated by luminal nucleotides could be a host defense reaction (Leipziger 2003). In this study, the ATP-induced ISc was comparable between distal colonic preparations from KCNMB2−/− and KCNMB2+/+ mice. Interestingly, ATP-induced ISc was increased in KCNMB2−/− mice compared to KCNMB2+/+ mice when the animals were fed an aldosterone-inducing diet for 4 days.

The observations that both basal and ATP-stimulated K+ conductance is intact in the colon from KCNMB2−/− mice could potentially be explained either by expression of other K+ channels or by intact KCa1.1 channel activity in the absence of the β2-subunit. It has been suggested that other K+ channels, in addition to KCa1.1, may contribute to distal colonic K+ secretion in other mammalian species (Zhang et al. 2012). However, in the current study, apical application of the specific KCa1.1 channel blocker IBTX, indisputably demonstrated that distal colonic K+ secretion also is mediated by KCa1.1 channels in the absence of the KCNMB2 subunit. This is in line with numerous previous studies that finds KCa1.1 channel as the only important apical conductance for murine colonic K+ secretion (Sausbier et al. 2006; Sorensen et al. 2008, 2010). One could speculate that the physiological function of the KCa1.1 channel, in the absence of KCNMB2−/− subunits, was supported by one
or some of the other β-subunit (β1, β3 or β4). We used semi-quantitative RT-PCR to investigate the transcription level of the remaining β-subunits. The β1, β3 or β4 subunits were all virtually undetectable in colonic epithelia from both KCNMB2+/+ and KCNMB2−/− mice. This raises the question of what supports distal colonic KCa1.1 activity in the absence of the β2-subunit. A recently identified family of γ-subunits, which are able to substantially modulate the KCa1.1 channel in a manner similar to the β-subunits (Yan and Aldrich 2010, 2012) may provide an answer to this question. We detected high mRNA expression of the γ1-subunit in the distal colonic epithelium of both KCNMB2+/+ and KCNMB2−/− mice. These data indicate that murine distal colonic KCa1.1 channel activity could be regulated by both the β2- and the γ1-subunit. Expression of a γ1-subunit of the KCa1.1 channel might contribute significantly to the physiological function of the KCa1.1 channel. While increasing the Ca2+-sensitivity of the KCa1.1 channel, the β2-subunit also causes rapid inactivation and reduced surface expression of the KCa1.1 channel (Bentrop et al. 2001; Zarei et al. 2007). In the absence of β2-subunit one could expect less inactivation and higher surface expression of the KCa1.1 channel, which potentially could explain why KCa1.1 activity was increased in the KCNMB2−/− mice. It is unresolved whether β- and γ-subunits of KCa1.1 can regulate the channel simultaneously, although heterologous expression experiments indicate that β- and γ-subunits interact with the α-subunit at the same domain, and that β-subunits may outcompete γ-subunits for interaction with α-subunits when both are present (Yan and Aldrich 2012). However, the γ1-subunit has been demonstrated to participate in regulation of the KCa1.1 channel in isolated smooth muscle arterial cells (Evanson et al. 2014), in which the KCa1.1 channels have previously been thought to be composed of α- and β1-subunits (Brenner et al. 2000b; Plüger et al. 2000).

To investigate the role of the γ1-subunit in murine distal colonic K+ secretion, we acquired a γ1-knock-out mouse (LRRB26+/−). Surprisingly, LRRB26 ablation led to a perinatal lethal phenotype. That a deletion of a KCa1.1 channel γ1-subunit resulted in a lethal phenotype was unexpected since mice with a global deletion of the pore-forming α-subunit of the KCa1.1 channel complex are viable, but display severe motoric, neurologic, and endocrine disturbances (Ruttiger et al. 2004; Sausbier et al. 2004, 2005; Engel et al. 2006; Typlt et al. 2013). This suggests that the LRRB26 gene or gene product could have other cellular functions in addition to being an auxiliary subunit of the KCa1.1 channel complex. The lethal phenotype of the LRRB26−/− mice, rendered us unable to perform experiments to address a possible role of the γ1-subunit in the distal colonic K+ secretion.

In summary, KCNMB2−/− mice did not display disturbed distal colonic K+ secretion, indicating that the β2-subunit of the KCa1.1 channel is not essential to support distal colonic K+ secretion. In fact, distal colonic K+ secretion was increased in KCNMB2−/− mice compared to KCNMB2+/+ mice when animals were fed a high K+/low Na+ diet. The intact distal colonic KCa1.1 activity in KCNMB2−/− mice might have been supported by the γ1-subunit of the KCa1.1 channel, although the role of this subunit in distal colonic K+ secretion needs to be studied in a tissue specific LRRB26 knock-out model.

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Conflict of Interest

There are no conflicts of interest to disclose.

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