

ORIGINAL RESEARCH

Intact colonic $K_{Ca}1.1$ channel activity in $KCNMB2$ knockout mice

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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 Ca^{2+} -activated K^+ channel ($K_{Ca}1.1$) 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 $K_{Ca}1.1$ channel is not yet fully established. This study investigates colonic K^+ secretion in a global knockout mouse of the $K_{Ca}1.1$ - β 2-subunit ($KCNMB2^{-/-}$), which apparently is the only β -subunit of the colonic enterocyte $K_{Ca}1.1$ channel. We can report that: (1) Neither $K_{Ca}1.1$ α - nor the remaining β -subunits were compensatory transcriptionally regulated in colonic epithelia of $KCNMB2^{-/-}$ mice. (2) Colonic epithelia from $KCNMB2^{-/-}$ mice displayed equal basal and ATP-induced $K_{Ca}1.1$ -mediated K^+ conductance as compared to $KCNMB2^{+/+}$. (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 $K_{Ca}1.1$ channel iberitoxin in both $KCNMB2^{+/+}$ and $KCNMB2^{-/-}$ mice. Recently a novel family of auxiliary γ -subunits of the $K_{Ca}1.1$ channel has been described. (5) We detected the γ 1-subunit (LRRC26) mRNA in colonic epithelia. To investigate the physiological role of the γ 1-subunit of $K_{Ca}1.1$ 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 β 2-subunit of $K_{Ca}1.1$ channel complex despite no additional compensatory induction of $K_{Ca}1.1$ 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 $K_{Ca}1.1$ channel complexes (Sausbier et al. 2006). The $K_{Ca}1.1$ channel consists of pore-forming α -subunits and

regulatory β -subunits, of which there are four different isoforms (β 1–4) (Orio et al. 2002; Lippiat et al. 2003). The different β -subunit isoforms confer different activation and inactivation properties to the $K_{Ca}1.1$ channel, but all increases the Ca^{2+} 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 $K_{Ca}1.1$ channels is ubiquitously expressed. Tissue specific coexpression of the different β -subunits is an important way, in which $K_{Ca}1.1$ channel function can be tailored to cell type specific functions. In the murine distal colonic

epithelium, K_{Ca}1.1 channels are composed exclusively of α - and β 2-subunits (Sorensen et al. 2008).

The β 2-subunit is unique among the K_{Ca}1.1 β -subunits as it inflicts both activating and inactivating properties to the K_{Ca}1.1 channel (Lippiat et al. 2003; Orio and Latorre 2005). Coexpression of α - and β 2-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; Orio and Latorre 2005). On the other hand, β 2-subunits mediate rapid inactivation of the K_{Ca}1.1 channel via an N-terminal ball-and-chain domain on the β 2-subunits (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 β 2-subunit (Zarei et al. 2007). Because of the dual nature of β 2-mediated regulation of the K_{Ca}1.1 channel, it is difficult to predict the overall effect of removal of β 2-regulation of K_{Ca}1.1 channels in the murine distal colon.

In this study, we use a mouse model with a global deletion of the β 2-subunit (KCNMB2^{-/-}) of the K_{Ca}1.1 channel to investigate the functional role of the β 2-subunit in distal colonic K⁺ secretion. In accordance with our previous work, we found that murine distal colonic epithelial cells express α - and β 2-subunits (Sorensen et al. 2008). K_{Ca}1.1 channels that are composed of α -subunits only require substantial membrane depolarization to open (Orio et al. 2002; Lippiat et al. 2003). This raises the question as to how distal colonic K_{Ca}1.1 channel activity is supported in KCNMB2^{-/-} mice. One answer could be provided by the family of γ -subunits (γ 1-4, encoded by the genes LRRC26, LRRC52, LRRC55, and LRRC38, respectively) recently described for the K_{Ca}1.1 channel. These γ -subunits similar to the β -subunits modulate K_{Ca}1.1 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 γ 1-subunits support K_{Ca}1.1 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^{-/-} littermates. 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'-ATATTGGAACTGCCCTTCTAAATCCC-3', WT-Rev: 5'-GTTCTCCCTCAGATCTAGGTATAGCAGC-3', KO-Fw: 5'-GCAGCCTCTGTTCCACATACACTTCA-3', and KO-Rev: 5'-TACATGACTAGCTCTCCCAGTGTCC-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), LRRC26 (Mm00525100_g1), LRRC38 (Mm01718430_m1), LRRC52 (Mm04204090_m1), LRRC55 (Mm02600578_m1), HPRT (Mm01545399_m1), and β -actin (Mm00607939_s1). Relative mRNA expression was calculated using the $2^{-\Delta C_t}$ -method (Schmittgen and Livak 2008), the average C_t value for HPRT and β -actin was used as reference C_t (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 (V_{te}) was measured continuously. The transepithelial resistance was calculated from the size of voltage deflections induced by injection of current pulses of 25 μ A with a duration of 1 sec (ΔV_{te}). These current pulses were injected continuously with a frequency of 0.2 Hz. Using Ohm's law, the equivalent short circuit current (I'_{sc}) was calculated from the transepithelial voltage and resistance. Upon mounting of the tissue in the Ussing chambers, 5 μ mol/L of indomethacin was added to both the apical and basolateral compartments and 1 μ mol/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 μ mol/L of amiloride was added to the apical compartment to inhibit ENaC-mediated Na⁺ transport. Five minutes after amiloride application, K_{Ca}1.1 channel activity was quantified as the change in I'_{sc} 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 K_{Ca}1.1 channel activity in the distal colon as the K_{Ca}1.1 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 I'_{sc} in distal colonic epithelia from $KCNMB2^{-/-}$ mice was in fact mediated by the K_{Ca}1.1 channel. Due to the size of IBTX (4230 Da) and the localization of K_{Ca}1.1 channels to the apical membrane of mucus covered distal colonic crypts (Sorensen et al. 2011), IBTX diffusion to the site of the K_{Ca}1.1

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 nmol/L luminal IBTX as the Ba²⁺ sensitive short circuit current 30 min after application of IBTX. K_{Ca}1.1 channel activity can also be transiently stimulated by application of nucleotides to the apical compartment (Matos et al. 2005). ATP-induced K_{Ca}1.1 activation was studied by application of 100 μ mol/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-gluconate, 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 K_{Ca}1.1 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 K_{Ca}1.1 channel in preparations of distal colonic epithelium. We confirmed our previous observation (Sorensen et al. 2008) that only the β 2 subunit is expressed at a significant level in the tissue (Fig. 1). β 2 mRNA was undetectable in preparations from

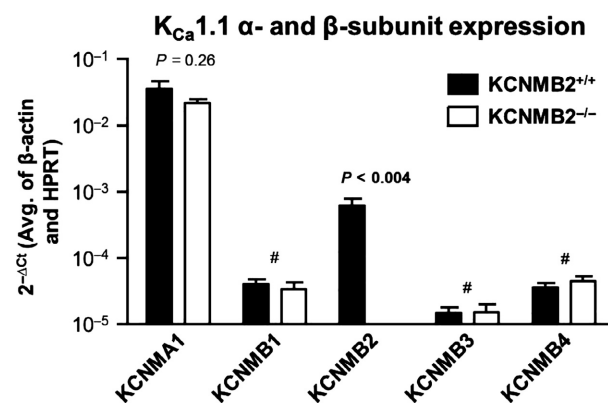


Figure 1. mRNA expression of BK channel α - and β -subunits in isolated colonic crypts from $KCNMB2^{+/+}$ and $KCNMB2^{-/-}$ mice relative to reference genes (β -actin and HPRT). $KCNMA1$ ($C_t \sim 26$) and $KCNMB2$ ($C_t \sim 30$) were detectable in crypts from $KCNMB2^{+/+}$. In crypts from $KCNMB2^{-/-}$ mice mRNA of $KCNMB2$ was not detectable whereas mRNA from crypts of $KCNMA1$ similar to $KCNMB2^{+/+}$. # denotes that $KCNMB1$, $KCNMB3$ and $KCNMB4$ were detectable but in irrelevant quantities ($C_t > 38$). Data are shown as means \pm SEM, $n = 6$ in all measurements.

$KCNMB2^{-/-}$ mice. No compensatory upregulation was observed in either the α -subunit or in any of the remaining β -subunits, although mRNA expression of β_1 , β_3 , and β_4 was too low ($C_t > 38$) to allow quantitative comparison of expression between genotypes.

Increased aldosterone stimulated colonic $K_{Ca}1.1$ activity in $KCNMB2^{-/-}$ mice

The effect of $KCNMB2$ ablation on distal colonic $K_{Ca}1.1$ channel activity was investigated in Ussing chambers. Basal $K_{Ca}1.1$ channel activity was quantified as Ba^{2+} -sensitive I'_{sc} and measured to be $9.2 \pm 1.0 \mu A cm^{-2}$ ($n = 13$) in $KCNMB2^{+/+}$ preparations and $12.2 \pm 1.1 \mu A cm^{-2}$ ($n = 13$) in $KCNMB2^{-/-}$ preparations (Fig. 2A, B and E). Colonic $K_{Ca}1.1$ 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 $K_{Ca}1.1$ channel activity between $KCNMB2^{+/+}$ and $KCNMB2^{-/-}$ mice might be more visible if the K^+ secretion was stimulated by an aldosterone-inducing diet for 4 days. This maneuver increased plasma aldosterone and distal colonic ENaC activity, the latter measured as amiloride sensitive I'_{sc} (data presented in table 1). Interestingly, the $K_{Ca}1.1$ channel activity was markedly increased in distal colonic epithelia from $KCNMB2^{-/-}$ mice, compared to $KCNMB2^{+/+}$ mice, when fed an aldosterone-inducing high K^+ /low Na^+ diet for 4 days. The Ba^{2+} -sensitive I'_{sc} was higher in the $KCNMB2^{-/-}$ preparations ($28.3 \pm 2.4 \mu A cm^{-2}$ [$n = 13$]) when compared with the

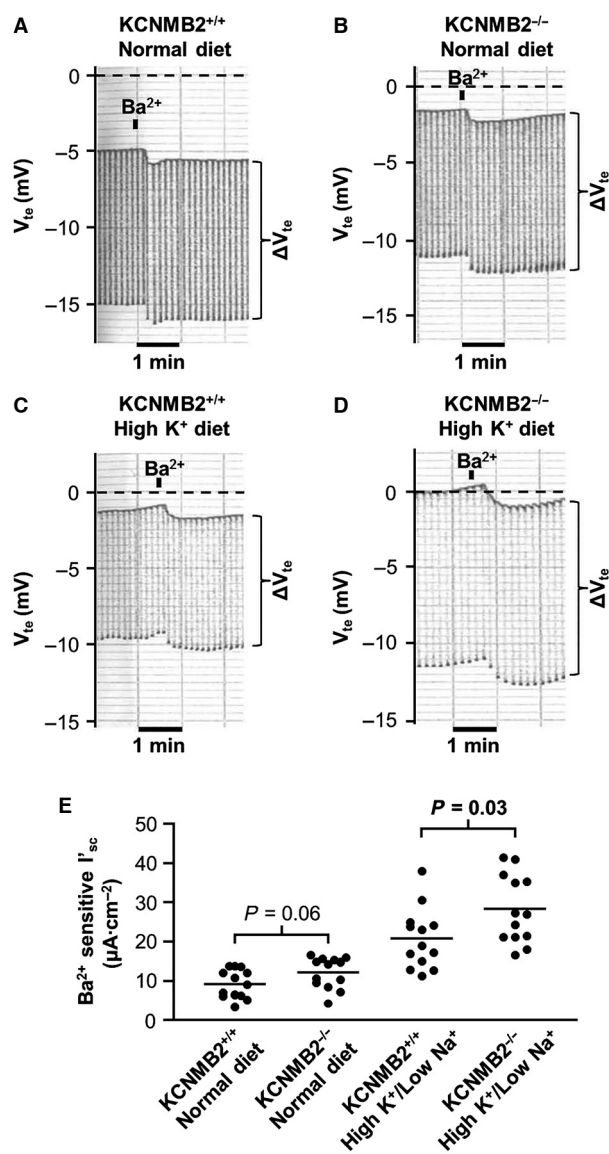


Figure 2. Barium sensitivity in isolated colonic mucosa from $KCNMB2^{+/+}$ and $KCNMB2^{-/-}$ mice. (A–D) shows original representative recordings of barium-sensitive transepithelial voltages (V_{te}) and resistance (ΔV_{te}) in tissues from $KCNMB2^{+/+}$ and $KCNMB2^{-/-}$ mice kept on control or aldosterone-augmenting diets. (E) summarizes the calculated Ba^{2+} -sensitive short circuit currents (I'_{sc}) in each of the four experimental groups. Horizontal lines represent mean values, $n = 13$ in each group. Note that Ba^{2+} -sensitive I'_{sc} is similar in all genotypes on control diet but statistically significantly augmented in the $KCNMB2^{-/-}$ as compared to $KCNMB2^{+/+}$ on the aldosterone-augmenting diet.

$KCNMB2^{+/+}$ preparations ($20.8 \pm 2.1 \mu A cm^{-2}$ [$n = 13$]) from animals kept for 4 days on the aldosterone-inducing high K^+ /low Na^+ diet (Fig. 2C, D and E). In summary, Ba^{2+} -sensitive baseline colonic $K_{Ca}1.1$ activity is not altered by deletion of $KCNMB2$ under normal dietary conditions,

Table 1. Plasma aldosterone and distal colonic amiloride sensitive I'_{sc} in KCNMB2^{+/+} and KCNMB2^{-/-} kept 4 days on an aldosterone-inducing diet.

Mouse genotype	Control diet		Aldosterone-inducing diet	
	KCNMB2 ^{+/+}	KCNMB2 ^{-/-}	KCNMB2 ^{+/+}	KCNMB2 ^{-/-}
Plasma aldosterone (pg/mL)	343 ± 39 (n = 6)	596 ± 73 (n = 6) ²	1756 ± 265 (n = 8) ¹	1850 ± 217 (n = 10) ¹
Amiloride-sensitive I'_{sc} (μA cm ⁻²)	-19.6 ± 1.9 (n = 14)	-20.3 ± 3.0 (n = 6)	-389.0 ± 46.0 (n = 8) ¹	-569.4 ± 77.3 (n = 10) ¹

¹*P* < 0.05 between same genotype on different diet.²*P* < 0.05 between genotypes on same diet.

but is augmented in mice subjected to simultaneous dietary K⁺ loading and Na⁺ depletion.

Ba²⁺-sensitive short circuit current is inhibited by specific K_{Ca}1.1 channel blocker

To verify whether the Ba²⁺-sensitive I'_{sc} in KCNMB2^{-/-} mice was in fact mediated by the K_{Ca}1.1 channel, we performed Ussing chamber experiments, in which 120 nmol/L of the specific K_{Ca}1.1 channel inhibitor IBTX was added to the luminal compartment. Thirty minutes after addition of IBTX or vehicle, BaCl₂ (5 mmol/L) was added to the luminal perfusate to assess any Ba²⁺-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 Ba²⁺-sensitive I'_{sc} of 13.2 ± 2.0 μA cm⁻² (n = 6) in the KCNMB2^{+/+} vehicle time controls, whereas Ba²⁺-sensitive I'_{sc} was reduced to 3.7 ± 2.3 μA 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 μA cm⁻² (n = 6) in the vehicle time controls and 0.7 ± 2.6 μA cm⁻² (n = 6) in preparations pretreated with IBTX (Fig. 3). These data indicate that no additional K⁺ conductance other than the K_{Ca}1.1 channel contributes to Ba²⁺-sensitive I'_{sc} in KCNMB2^{-/-} mice.

ATP-induced K_{Ca}1.1 activity was higher in colon from KCNMB2^{-/-} mice at high plasma aldosterone

K_{Ca}1.1 channel activity is stimulated by increased intracellular Ca²⁺ concentration ([Ca²⁺]_i). The nucleotides ATP and UTP are known to cause reliable, transient increase in [Ca²⁺]_i in colonic epithelia via luminal P2Y receptors (Matos et al. 2005). Addition of ATP (100 μmol/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 K_{Ca}1.1 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 μA cm⁻² (n = 6) in the KCNMB2^{+/+} and 31.2 ± 4.8 μA cm⁻² (n = 6) in the KCNMB2^{-/-}) (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 μA cm⁻² [n = 8] and 15.6 ± 4.3 μA cm⁻² [n = 8], respectively, Fig. 4C, D and E).

Expression of K_{Ca}1.1 γ-subunits in the distal colonic epithelium

A new family of K_{Ca}1.1 channel γ-subunits, which modulate K_{Ca}1.1 channel activity similarly to the β-subunits, has recently been described, and mRNA expression of some of these γ-subunits has been detected in whole human colon (Yan and Aldrich 2012). It is possible that the intact K_{Ca}1.1 channel activity in KCNMB2^{-/-} mice could be attributed to expression of one or more of these γ-subunits. We therefore performed semi-quantitative rt-PCR to determine whether any of the γ-subunits were expressed in the murine distal colonic epithelium. We detected high mRNA expression of γ1 (LRRC26), whereas mRNA expression of γ2, γ3 and γ4 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 γ-subunits between KCNMB2^{+/+} and KCNMB2^{-/-} mice (Fig. 5).

Post-natal lethality of LRRC26 mice

To investigate whether the γ1-subunit supports K_{Ca}1.1 channel activity in the distal colon, we acquired a knock-out mouse for the γ1-subunit (LRRC26^{-/-}). In total, we genotyped 77 pups from 10 litters at the time of weaning

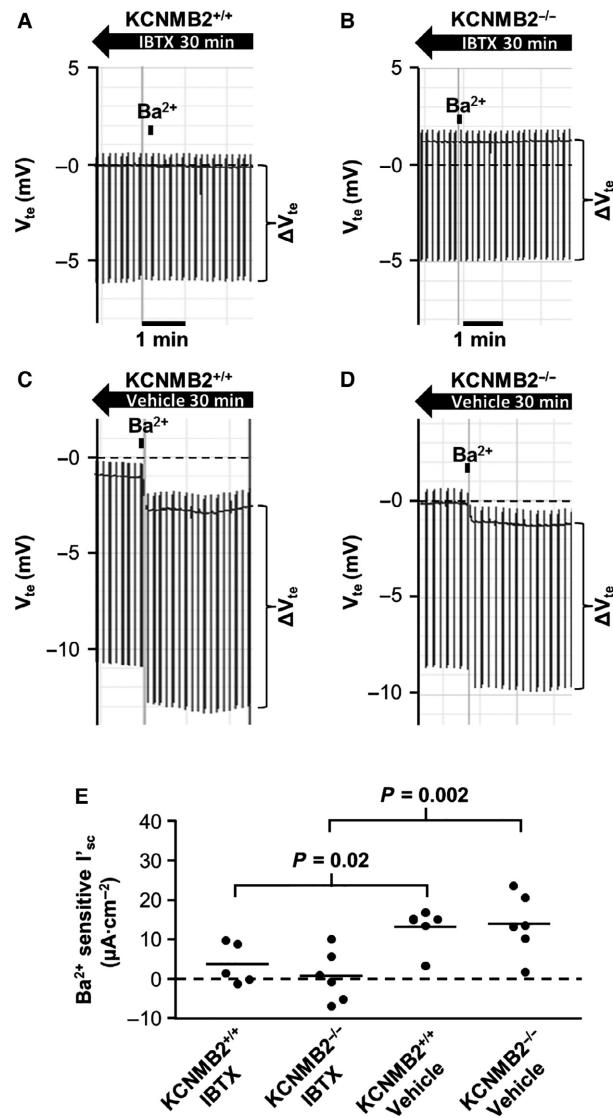


Figure 3. Barium sensitivity in isolated colonic mucosa from KCNMB2^{+/+} and KCNMB2^{-/-} mice pretreated 30 min with luminal (120 nmol/L) iberiotoxin (IBTX) or vehicle. (A–D) show original representative recordings of Ba²⁺-sensitive transepithelial voltages (V_{te}) and resistance (ΔV_{te}) in tissues, pretreated with luminal IBTX or vehicle, from KCNMB2^{+/+} and KCNMB2^{-/-} mice. (E) summarizes the calculated Ba²⁺-sensitive short circuit currents (I_{sc}) in each of the four experimental groups. Horizontal lines represent mean values, $n = 6$ in each group. Note that Ba²⁺-sensitive I_{sc} is almost abolished after pretreatment with IBTX.

(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

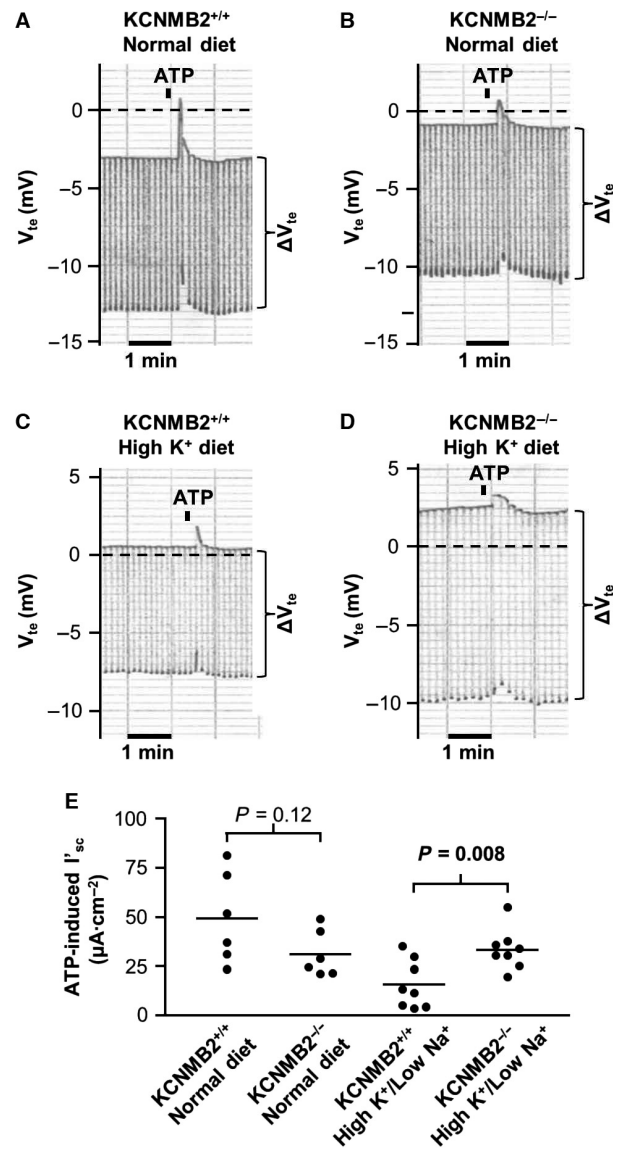


Figure 4. Effects of ATP stimulated on isolated colonic mucosa from KCNMB2^{+/+} and KCNMB2^{-/-} mice. (A–D) shows original representative recordings of luminal ATP effects on transepithelial voltages (V_{te}) and resistance (ΔV_{te}) in tissues from KCNMB2^{+/+} and KCNMB2^{-/-} mice kept on control or aldosterone-augmenting diets. (E) summarizes the calculated ATP induced peak in short circuit currents (I_{sc}) in each of the four experimental groups. Horizontal lines represent mean values, $n = 6-7$ in each group.

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

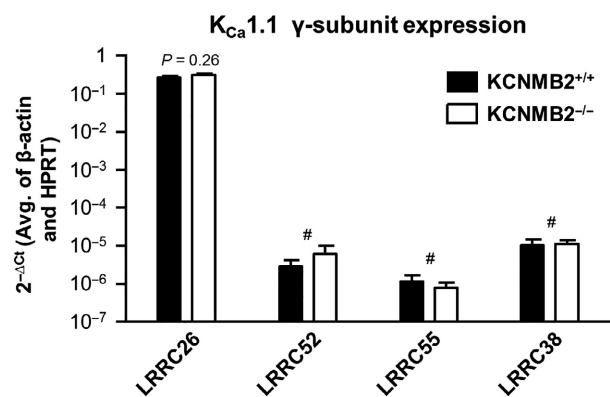


Figure 5. mRNA expression of BK channel γ -subunits (LRRC) in isolated colonic crypts from KCNMB2^{+/+} and KCNMB2^{-/-} mice relative to reference genes (β -actin and HPRT). LRRC26 was the only subunit detectable in relevant quantities ($C_t < 25$) in isolated colonic crypts. The level of LRRC26 mRNA was not dependent on the KCNMB2 gene. The remaining LRRC subunits were only detectable at very high C_t values. # denotes that mRNA was detectable but in irrelevant quantities ($C_t > 38$). Data are shown as means \pm SEM, $n = 6$ in all measurements.

were 5 LRRC26^{+/+}, 8 LRRC26^{+/-}, and 7 LRRC26^{-/-} (Fig. 6A). These data indicate that LRRC26 genotypes, as expected, shows 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 K_{Ca}1.1 channel activity. We confirm our observation, published in 2008, that the β 2-subunit of the K_{Ca}1.1 channel is the only K_{Ca}1.1 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 I'_{sc} ,

which was equal in size to that in KCNMB2^{+/+} preparations. In preparations from mice kept on an aldosterone-inducing diet, the Ba²⁺-sensitive I'_{sc} was increased in both genotypes, and this to even higher values in the KCNMB2^{-/-} tissues than in KCNMB2^{+/+} tissues.

On the physiological level, K_{Ca}1.1-mediated colonic K⁺ secretion is tightly regulated. A number of different receptor agonist acts 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 K_{Ca}1.1 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 I'_{sc} was comparable between distal colonic preparations from KCNMB2^{-/-} and KCNMB2^{+/+} mice. Interestingly, ATP-induced I'_{sc} 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 K_{Ca}1.1 channel activity in the absence of the β 2-subunit. It has been suggested that other K⁺ channels, in addition to K_{Ca}1.1, 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 K_{Ca}1.1 channel blocker IBTX, indisputably demonstrated that distal colonic K⁺ secretion also is mediated by K_{Ca}1.1 channels in the absence of the KCNMB2 subunit. This is in line with numerous previous studies that finds the K_{Ca}1.1 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 K_{Ca}1.1 channel, in the absence of KCNMB2^{-/-} subunits, was supported by one

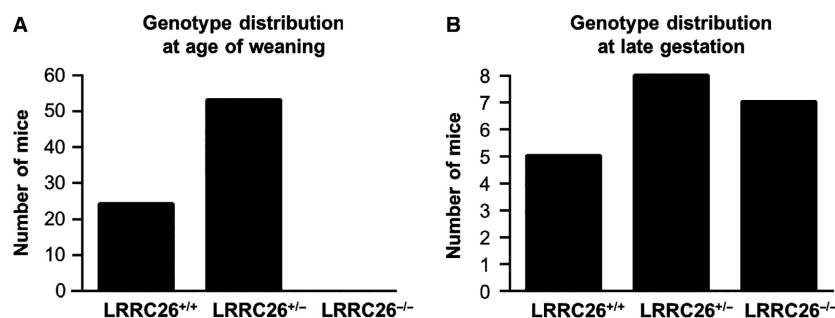


Figure 6. Genotype distribution in LRRC26 mice. (A) Age of weaning (~3 weeks). (B) late gestation (~day 18).

or some of the other β -subunit ($\beta 1$, $\beta 3$ or $\beta 4$). We used semi-quantitative rt-PCR to investigate the transcription level of the remaining β -subunits. The $\beta 1$, $\beta 3$ or $\beta 4$ subunits were all virtually undetectable in colonic epithelia from both KCNMB2^{-/-} and KCNMB2^{+/+} mice. This raises the question of what supports distal colonic K_{Ca}1.1 activity in the absence of the $\beta 2$ -subunit. A recently identified family of γ -subunits, which are able to substantially modulate the K_{Ca}1.1 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 $\gamma 1$ -subunit in the distal colonic epithelium of both KCNMB2^{+/+} and KCNMB2^{-/-} mice. These data indicate that murine distal colonic K_{Ca}1.1 channel activity could be regulated by both the $\beta 2$ - and the $\gamma 1$ -subunit. Expression of a $\gamma 1$ -subunit of the K_{Ca}1.1 channel might contribute significantly to the physiological function of the K_{Ca}1.1 channel. While increasing the Ca²⁺-sensitivity of the K_{Ca}1.1 channel, the $\beta 2$ -subunit also causes rapid inactivation and reduced surface expression of the K_{Ca}1.1 channel (Bentrop et al. 2001; Zarei et al. 2007). In the absence of $\beta 2$ -subunit one could expect less inactivation and higher surface expression of the K_{Ca}1.1 channel, which potentially could explain why K_{Ca}1.1 activity was increased in the KCNMB2^{-/-} mice. It is unresolved whether β - and γ -subunits of K_{Ca}1.1 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 out-compete γ -subunits for interaction with α -subunits when both are present (Yan and Aldrich 2012). However, the $\gamma 1$ -subunit has been demonstrated to participate in regulation of the K_{Ca}1.1 channel in isolated smooth muscle arterial cells (Evanson et al. 2014), in which the K_{Ca}1.1 channels have previously been thought to be composed of α - and $\beta 1$ -subunits (Brenner et al. 2000b; Pluger et al. 2000).

To investigate the role of the $\gamma 1$ -subunit in murine distal colonic K⁺ secretion, we acquired a $\gamma 1$ -knock-out mouse (LRRC26^{-/-}). Surprisingly, LRRC26 ablation led to a perinatal lethal phenotype. That a deletion of a K_{Ca}1.1 channel $\gamma 1$ -subunit resulted in a lethal phenotype was unexpected since mice with a global deletion of the pore-forming α -subunit of the K_{Ca}1.1 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 LRRC26 gene or gene product could have other cellular functions in addition to being an auxiliary subunit of the K_{Ca}1.1 channel complex. The lethal phenotype of the LRRC26^{-/-} mice, rendered us

were unable to perform experiments to address a possible role of the $\gamma 1$ -subunit in the distal colonic K⁺ secretion.

In summary, KCNMB2^{-/-} mice did not display disturbed distal colonic K⁺ secretion, indicating that the $\beta 2$ -subunit of the K_{Ca}1.1 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 K_{Ca}1.1 activity in KCNMB2^{-/-} mice might have been supported by the $\gamma 1$ -subunit of the K_{Ca}1.1 channel, although the role of this subunit in distal colonic K⁺ secretion needs to be studied in a tissue specific LRRC26 knock-out model.

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Conflict of Interest

There are no conflicts of interest to disclose.

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