Cloning and functional expression of the mouse epithelial sodium channel

Yoon J. Ahn, David R. Brooker, Farhad Kosari, Brian J. Harte, Jinqiong Li, Scott A. Mackler, and Thomas R. Kleyman

Departments of Medicine and Physiology, University of Pennsylvania, and
Veterans Affairs Medical Center, Philadelphia, Pennsylvania 19104-6144

Ahn, Yoon J., David R. Brooker, Farhad Kosari, Brian J. Harte, Jinqiong Li, Scott A. Mackler, and Thomas R. Kleyman. Cloning and functional expression of the mouse epithelial sodium channel. Am. J. Physiol. 277 (Renal Physiol. 46): F121–F129, 1999.—The epithelial sodium channel (ENaC) plays a major role in the transepithelial reabsorption of sodium in the renal cortical collecting duct, distal colon, and lung. ENaCs are formed by three structurally related subunits, termed α-, β-, and γENaC. We previously isolated and sequenced cDNAs encoding a portion of mouse α-, β-, and γENaC. These cDNAs were used to screen an oligo-dT-primed mouse kidney cDNA library. Full-length αmENaC and partial-length α- and γmENaC cDNAs were isolated. Full-length α- and γmENaC cDNAs were subsequently obtained by 5′-rapid amplification of cDNA ends (5′-RACE) PCR. Injection of mouse α-, β-, and γmENaC cDNAs into Xenopus oocytes led to expression of amiloride-sensitive currents (K1 = 103 nM), Na+-selective currents with a single-channel conductance of 4.7 pS. Northern blots revealed that α-, β-, and γmENaC were expressed in lung and kidney. Interestingly, αmENaC was detected in liver, although transcript sizes of 9.8 kb and 3.1 kb differed in size from the 3.2-kb message observed in other tissues. A partial cDNA clone was isolated from mouse liver by 5′-RACE PCR. Its sequence was found to be nearly identical to αmENaC. To begin to identify regions within αmENaC that might be important in assembly of the native heterodimeric channel, a series of functional experiments were performed using a construct of αmENaC encoding the predicted cytoplasmic NH2-terminus. Construction of wild-type α-, β-, and γmENaC with the intracellular NH2-terminus of αmENaC abolished amiloride-sensitive currents in Xenopus oocytes, suggesting that the NH2-terminus of αmENaC is involved in subunit assembly, and when present in a 10-fold excess, plays a dominant negative role in functional ENaC expression.

cloning; Xenopus oocytes; structure-function relationship

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suggesting that the NH₂ terminus of αmENaC is involved in subunit assembly.

MATERIALS AND METHODS

Reagents were purchased from Sigma Chemical (St. Louis, MO), unless otherwise specified. For molecular biology protocols, standard procedures were followed (2, 41, 57). DNA sequencing and oligonucleotide syntheses were performed by the University of Pennsylvania DNA Core Facility.

Generation of probes and library screen. cDNAs partially encoding mouse α-, β-, and γENaC were isolated as previously described (6). These cDNAs were radio labeled by random priming (Prime-It II random primer labeling kit; Stratagene, La Jolla, CA) with [α-32P]dATP (ICN, Costa Mesa, CA). A mouse kidney cDNA library cloned into the Lambda Uni-ZAP XR vector (Stratagene) and transformed bacteria were plated, and colonies were transferred to Hybond-N nylon filters (Amersham, Arlington Heights, IL). For primary screening, the filters were hybridized overnight to the labeled probes in hybridization solution (6× standard saline citrate (SSC), 20 mM NaH₂PO₄, 0.5% (wt/vol) SDS, and 500 µg/ml denatured, sonicated salmon sperm DNA) at 55°C. Filters were washed with 2× SSC + 0.1% SDS at room temperature, twice with 1× SSC + 0.1% SDS at 50°C, and once with 0.1× SSC + 0.1% SDS at 50°C. Filters were exposed overnight to Kodak X-Omat AR film at −70°C. Positive colonies on duplicate filters were selected and rescreened. Colonies were isolated by sib selection, subjected to plasmid rescue, excision to determine insert size, and partial nucleotide sequencing of the 5’ end of the inserts.

Generation of full-length α- and γmENaC subunits. As no full-length αmENaC or γmENaC clones were obtained in the library screen, 5’ rapid amplification of cDNA ends (5’- RACE) PCR using gene-specific antisense primers based on known sequence (5’-TGGAAAGACATCCAGAGATT-3’ for α- and 5’-CCACCAGTTTCTCTTGACTCAT-3’ for γmENaC) was performed to obtain the missing upstream fragments of the α- and γmENaC subunits. PCR reaction conditions were as follows: initial denaturation at 94°C for 2 min, 30 amplification cycles (94°C for 10 s, 55°C for 20 s, 68°C for 2 min), and final elongation at 68°C for 10 min. The PCR products were subcloned into pCR2 (Invitrogen, Carlsbad, CA). The upstream (PCR products) and downstream (cDNAs from library screen) fragments of α- and γmENaC were ligated following restriction enzyme digestion to generate the full-length α- or γmENaC cDNAs. Full-length clones were sequenced in both directions by the method of Sanger et al. (42).

Cloning of NH₂-terminal αmENaC and ectodomain αmENaC. The cDNA residues encoding the NH₂-terminal domain of αmENaC (corresponding to amino acid residues 1-581) were PCR amplified and subcloned into pBS SK (−) (Stratagene). cDNA encoding the ectodomain of αmENaC (corresponding to amino acid residues Y166-P568) was PCR amplified and subcloned into pBK-CMV (Stratagene). Sequences were confirmed by Sanger dideoxynucleotide sequence analysis.

Northern blots. A commercial mouse multiple tissue Northern blot containing equal quantities (2 µg) of purified poly(A)⁺ RNA in each lane was used (Clontech, Palo Alto, CA) to examine mENaC tissue distribution. cDNA fragments of αmENaC (G1365-T1755), βmENaC (T811-C1018), γmENaC (G958-C1205), and mouse β-actin were 32P labeled (14) and individually hybridized with the membrane overnight at 50°C following the manufacturer’s protocol. The blots were washed at high stringency (final wash, 0.1× SSC at 65°C) and exposed to film or to a phosphor screen for imaging (Molecular Dynamics, Sunnyvale, CA).

Oocyte expression and electrophysiology. cRNAs were generated from mENaC cDNA inserts in pBS SK (−) or pBK-CMV vector using a T3 cRNA synthesis kit (m-MESSAGE MA-CHINE; Ambion, Austin, TX) following the manufacturer’s protocol. Prior to transcription all cDNA constructs were linearized by restriction digestion with Xho I except for γmENaC where Kpn I was used. Oocytes were isolated from Xenopus laevis females (Nasco, Fort Atkinson, WI), and stage V-VI oocytes were selected for collagenase treatment following standard protocols (57). Oocytes were injected with full-length mouse α-, β-, and γmENaC cRNAs at a concentration of 2 ng·subunit⁻¹·oocyte⁻¹. NH₂-terminal αmENaC cRNA was injected at concentrations of 20 ng, 6 ng, or 2 ng per oocyte. All cRNAs were injected in a volume of 50 nl·oocyte⁻¹. Following injection, oocytes were incubated at 18°C in modified Barth’s saline [88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 15 mM HEPES, 0.3 mM Ca(NO₃)₂, 0.41 mM CaCl₂, 0.82 mM MgSO₄, 10 µg/ml penicillin, 10 µg/ml streptomycin sulfate, 100 µg/ml gentamycin sulfate, and 10 µg/ml nystatin; pH 7.2] and then assayed 18–48 h postinjection. In selected experiments, oocytes were incubated in a low-Na⁺ modified Barth’s saline (the 88 mM NaCl was replaced with 88 mM KCl) to prevent the cells from loading with Na⁺ prior to voltage clamping. Whole cell currents were measured using the two-electrode voltage clamp technique (TEV) at a holding potential of −100 mV (with reference to bath) for 500 ms and then 450 ms at 0 mV. During recordings, oocytes were bathed in a sodium gluconate buffer [100 mM sodium gluconate, 2 mM KCl, 1.8 mM CaCl₂, 10 mM HEPES, 5 mM BaCl₂, 10 mM tetraethylammonium chloride (TEA-Cl), pH 7.2]. Cation selectivity measurements were performed in sodium gluconate or potassium gluconate (100 mM potassium gluconate, 2 mM KCl, 1.8 mM CaCl₂, 10 mM HEPES, 5 mM BaCl₂, and 10 mM TEA-Cl, pH 7.2) buffers. Amiloride-sensitive currents were determined by subtracting currents measured in oocytes perfused with sodium gluconate (or potassium gluconate) buffers supplemented with 100 µM amiloride from baseline currents in sodium gluconate (or potassium gluconate) buffers. TEV was performed under continuous flow (−4 ml/min) of buffers. Single-channel recordings were performed in the cell-attached configuration. All data were collected at room temperature and were filtered at 300 Hz. The applied voltage to the membrane patch represents the voltage deflection from the resting membrane potential. Inward Na⁺ currents were represented by downward deflections in single-channel recordings. Measurements of single-channel conductance were performed with a buffer containing 100 mM NaCl, 1.8 mM CaCl₂, 2 mM KCl, and 10 mM HEPES, pH 7.2, in the patch pipette and in the bath. Statistical analyses were performed with pCLAMP software (Axon Instruments) or MatLab (MathWorks).

Statistics. Results are expressed as means ± SE. Statistical significance was determined by Student’s t-test.

RESULTS

Cloning of full-length ENaC subunits. Labeled probes for α-, β-, and γENaC were synthesized as previously described (6) and used to screen mouse kidney oligo-dT primed cDNA lambda libraries. Primary screening yielded 8 positive colonies for αmENaC, 9 positive
colonies for βmENaC, and 12 positive colonies for γmENaC. All αmENaC, 2 βmENaC, and 11 γmENaC colonies were successfully isolated by sib selection, subjected to plasmid rescue, and excision to determine insert size, followed by partial nucleotide sequencing of the 5′ end of the inserts. Two full-length βmENaC clones were isolated (clones B1 and B2), but no full-length αmENaC or γmENaC clones were obtained (9). The αmENaC clone with the longest insert (clone A2) apparently lacked 978 residues of the 5′ end of the open-reading frame compared with rat αENaC (9). The γmENaC clones with the longest inserts (clones G5 and G11) appeared to lack 428 and 388 residues, respectively, at the 5′ end of the open-reading frame compared with γENaC (9). Clones A2, B1, and G5, and G11 were sequenced. The sequence of G11 was ~1 kb longer than both the γmENaC clone G5 and γENaC in the 3′ untranslated region (data not shown). On the basis of sequence analysis, this 1-kb addition likely represented a cloning artifact.

5′-RACE was performed to obtain cDNAs encoding the 5′ regions of α- and γmENaC. Sequence analyses confirmed that we had obtained the 5′ regions of α- and γmENaC. The RACE products and the partial-length α- and γmENaC cDNAs were subjected to restriction digestion and ligation to obtain full-length α- and γmENaC cDNAs.

The full-length mENaC clones were sequenced. The deduced amino acid sequences of the mouse α-, β-, and γENaCs were 95%, 96%, and 97% identical to rat α-, β-, and γENaC, respectively, and 87%, 88%, and 88% identical to human α-, β-, and γENaC. Sequence comparisons illustrate the high degree of identity between rat and mouse ENaC, as depicted in Fig. 1. Of particular interest was a 15-nucleotide insertion present in γmENaC encompassing residues T399-C413, which is absent in rat. This insertion did not occur in close proximity to defined rat genomic intron-exon junctions (55) and likely did not arise as a result of alternative mRNA splicing. PCR analysis of mouse genomic DNA using primers flanking the 15-bp insertion (sense 5′-GATGTTCAGAACTACCATCGGAAA-3′; antisense 5′-AAATCCCACCATTTTCTGGACTCATG-3′) was performed. The 235-bp PCR product was sequenced and confirmed that the 15-bp insertion represented a species-specific phenomenon at the genomic level.

Tissue distribution. Tissue distribution of α-, β-, and γmENaC was examined by Northern blot analyses. As expected, all three mENaCs were expressed in mouse lung and kidney, with the α-, β-, and γmENaC probes recognizing mRNAs of 3.7, 2.6, and 3.2 kb, respectively (Fig. 2). αmENaC recognized two mRNA species in mouse liver, of ~9.8 and 3.1 kb. Hybridization to liver mRNAs was observed under high-stringency conditions, but the signal was relatively weak. These results suggested that αmENaC is expressed in liver. To further confirm this observation, 5′-RACE PCR using a 3′ gene-specific primer (5′-CGGAAACCTGCGAATAACATGATG-3′) and a 5′ adaptor primer was performed on liver cDNA (Clontech). A PCR product of 702 bp was obtained. Its sequence was nearly identical to αmENaC (sequence not shown), providing additional evidence that αmENaC or an αmENaC isofrom is expressed in mouse liver.

Functional expression of mENaCs. The Xenopus oocyte expression system was used to examine the functional properties of mouse ENaCs. Whole cell amiloride-sensitive currents obtained in oocytes injected with α-, β-, and γmENaC cRNAs by the TEV technique are illustrated in Figs. 3 and 4. Amiloride inhibited the Na+ current with an IC50 of 103 ± 16 nM (Fig. 3, n = 7). The Na+/K+ selectivity ratio was greater than 80:1 at a holding potential of ~100 mV (Fig. 4, n = 5). Analyses of Na+ channel characteristics by cell-attached patch clamp revealed long open and closed times (on the order of seconds) (Fig. 5) and a linear current/voltage relationship with a slope conductance of 4.7 pS (Fig. 6, n = 4–6). These functional characteristics of mouse ENaCs are in agreement with the characteristics of the cloned rat, human, and X. laevis ENaCs (9, 31, 36).

Amino-terminal αmENaC has a dominant negative effect on ENaC expression in Xenopus oocytes. We examined whether coexpression of the NH2-terminal domain of αmENaC with wild-type α-, β-, and γmENaC would inhibit the formation of functionally competent channels in Xenopus oocytes. Coinjection of wild-type α-, β-, and γmENaC cRNAs with a 10-fold excess (weight basis) of αmENaC NH2-terminal mRNA in Xenopus oocytes inhibited amiloride-sensitive current compared with injection with wild-type cRNAs alone (Fig. 7, n = 20). Furthermore, this effect was dose dependent. A partial inhibition of amiloride-sensitive current was observed at coinjection ratios of 3:1 and 1:1 (Fig. 7, n = 6). Oocytes cojected with wild-type mENaC cRNAs and a 10-fold excess of a control cRNA encoding for the ectodomain of αmENaC produced Na+-selective currents commensurate with those seen in oocytes injected with wild type alone (Fig. 8, n = 7). These data suggest that the NH2 terminus of αmENaC is involved in subunit assembly, and when present in a 10-fold excess, has a dominant negative role in functional ENaC expression in Xenopus oocytes.

DISCUSSION

The epithelial sodium channel is a heteroligomeric protein comprising three homologous subunits, α-, β-, and γENaC (9). We obtained full-length cDNAs encoding mouse α-, β-, and γENaC. The deduced amino acid sequences of the mouse ENaCs are nearly identical to rat α-, β-, and γENaC, respectively (Fig. 1) (7, 9). Dagenais et al. (12) have reported a partial cDNA clone of mouse αENaC, corresponding to amino acid residues H4455-L558 of our full-length mouse αENaC. There are three residues that differ from our sequence near the extreme NH2 and COOH termini, which may reflect sequence polymorphisms. Regions within ENaCs that have defined functions are conserved. For example, each subunit has two putative membrane-spanning domains (M1 and M2) that are amphipathic and predicted to assume an α-helical structure (Fig. 1). A predominantly hydrophobic domain, previously termed H2 by Canessa and coworkers (9), precedes the second
membrane-spanning domain of each subunit and may contribute to the channel pore and selectivity filter (9, 37), as mutations within this region alter cation selectivity, amiloride-sensitivity, and single-channel conductance (29, 37, 44, 45, 60). The region between the two hydrophobic membrane-spanning domains comprising approximately two-thirds of the mass of each subunit is extracellular (8, 52). Sequence analysis of this large

![Fig. 1. Deduced amino acid sequences of mouse α-, β-, and γ-subunits of the epithelial sodium channel (ENaC). Sequence comparisons with rat ENaCs (7, 9) are included. Amino acid identity is indicated by a dash. Putative membrane-spanning domains (M1 and M2) are indicated by bold underscore.](http://ajprenal.physiology.org/)
extracellular domain reveals multiple N-linked glycosylation sites (6, 12, and 5 N-linked glycosylation sites for α-, β-, and γ-subunits, respectively) and cysteine-rich domains, features conserved between the three subunits as well as other members of the ENaC/mec/deg superfamily (11, 19). The relatively short cytoplasmic NH₂- and COOH-termini have consensus sites for phosphorylation by protein kinase A and protein kinase C. Recent studies support the notion that phosphorylation of the β- and γ-subunits of the channel may have a role in the regulation of the channel by forskolin, insulin, aldosterone, and phorbol esters (47). Proline-rich regions and PXY motifs have defined roles in the binding of α-spectrin and the ubiquitin ligase Nedd4 to the channel (40, 54). A gating domain has been identified within the NH₂ terminus of the α-subunit (22). These regions are conserved within the mouse ENaCs reported here.

Several groups have reported that the subunit stoichiometry of ENaC is α₂β₁γ₁ (15, 29), although one group has reported a subunit stoichiometry of α₃β₃γ₃ (51). When the three subunits are expressed in Xenopus oocytes, they oligomerize to form a channel with properties similar to that observed in native tissues (9, 19, 31). We also observed that mouse ENaCs, when expressed in Xenopus oocytes, are highly selective for sodium rich regions and PXY motifs have defined roles in the binding of α-spectrin and the ubiquitin ligase Nedd4 to the channel (40, 54). A gating domain has been identified within the NH₂ terminus of the α-subunit (22). These regions are conserved within the mouse ENaCs reported here.

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Fig. 2. Mouse α-, β-, and γENaC tissue distribution. A mouse multiple tissue Northern blot containing equal quantities (2 µg) of poly(A)¹ RNA per lane was hybridized consecutively with ³²P-labeled α-, β-, or γmENaC or mouse actin probes as described in the MATERIALS AND METHODS. Bound probe was visualized by autoradiography or phosphorimager. All three mENaCs were expressed in mouse lung and kidney, with the α-, β-, and γmENaC probes recognizing mRNAs of 3.7, 2.6, and 3.2 kb, respectively. Sk muscle, skeletal muscle.

Fig. 3. Expression of mouse α-, β-, and γENaC in oocytes: amiloride dose-response relationship. Oocytes were injected with α-, β-, and γmENaC cRNAs and maintained in modified Barth's saline. Currents were measured in oocytes bathed in sodium gluconate in presence of increasing concentrations of amiloride using the two-electrode voltage clamp technique with a holding potential of −100 mV. Currents shown were normalized to the current measured in absence of amiloride. IC₅₀ for amiloride was 10³ ± 16 nM (n = 7).

Fig. 4. Expression of mouse α-, β-, and γENaC in oocytes: whole cell current/voltage (I/V) relationships and cation selectivity. Oocytes were injected with α-, β-, and γmENaC cRNAs and maintained in a low-Na¹ modified Barth's solution. Currents were measured while varying the holding potential between −100 and +60 mV (20 mV steps), using the TEV technique. Oocytes were bathed in a buffer containing either sodium gluconate (r) or potassium gluconate (s) in presence or absence of 100 µM amiloride. Amiloride-sensitive currents are shown. Currents were normalized to the value obtained at a holding potential of −100 mV in Na¹ bath (n = 5).
(Na\textsuperscript{+} >> K\textsuperscript{+}) (Fig. 4), demonstrate slow gating kinetics (long open and closed times on the order of several seconds) (Fig. 5), low single-channel conductance (4.7 pS) (Fig. 6), and are blocked by amiloride with a $K_i$ of 103 nM (Fig. 3).

Liver $\alpha$mENaC gene product may serve as an amiloride-sensitive Na\textsuperscript{+} channel. Mouse ENaC mRNA was expressed in sodium-absorptive epithelia (Fig. 2), including kidney and lung. Mouse $\alpha$mENaC, like human $\alpha$ENaC (31), was expressed in liver. Interestingly, the $\beta$- and $\gamma$-subunits were not detected in liver. The expression of the $\alpha$-subunit alone raises the question of whether functional Na\textsuperscript{+} channels are expressed in the liver, either composed solely of $\alpha$-subunits or a heteroligomer of $\alpha$-subunits with related ENaC subunits that have yet to be identified. Na\textsuperscript{+}-conductive pathways have an important role in hepatocyte volume regulation (61).

Rat hepatocytes in confluent primary cultures respond
to hypertonic stress with a considerable increase in cell membrane Na\(^+\) conductance. These adaptations (the regulatory volume increase, the increases in Na\(^+\) conductance and intracellular Na\(^+\) concentration, as well as the activation of Na\(^+\)-K\(^+\)-ATPase) were completely blocked by 10 \(\mu\)M amiloride (61). At this concentration, amiloride had no effect on osmotically induced cell alkalization via Na\(^+\)/H\(^+\) exchange (61). These data imply that an amiloride-sensitive Na\(^+\) channel is expressed in hepatocytes and serves as the conduit for Na\(^+\) influx. Activation of Na\(^+\) channels, in conjunction with activation of Na\(^+\)-K\(^+\)-ATPase, results in increases in intracellular K\(^+\) and Na\(^+\), and these are the major ionic mechanisms responsible for regulatory volume increases in hepatocytes. Our observation that \(\alpha\)mENaC was expressed in liver suggested that \(\alpha\)ENaC (and possibly as yet unidentified related subunits) may function as a mechanosensitive cation channel and play an important role in hepatocyte cell volume regulation.

The question of whether ENaC is a mechanosensitive ion channel has been examined by several laboratories. Differing results have been reported. Channels composed of all three ENaC subunits were mechanosensitive when expressed in lipid bilayers (25). Xenopus oocytes expressing \(\alpha\), \(\beta\), and \(\gamma\)ENaC responded to cell swelling with either no change (3) or a decrease (26) in Na\(^+\) conductance and responded to cell shrinkage with an increase (26) or decrease (3) in Na\(^+\) conductance. Na\(^+\) channels in the collecting tubule responded in a variable manner to an increase in membrane tension by altering the hydrostatic pressure in a patch pipette (34); an increase in open probability was reported in 6 of 22 patches. Although controversy exists in the literature as to the effects of changes in cell volume on functional \(\alpha\), \(\beta\), and \(\gamma\)ENaC expression, these data do not exclude the possibility that channels formed by \(\alpha\)-subunits alone are mechanosensitive.

The biophysical properties of Na\(^+\) channels composed of \(\alpha\)-subunits differ from \(\alpha\), \(\beta\), and \(\gamma\)ENaC with regard to single-channel conductance and cation selectivity (9, 31, 32). Application of a hydrostatic pressure gradient across lipid bilayers increases the open probability of \(\alpha\)ENaC (4). When expressed in fibroblasts, \(\alpha\)ENaCs were activated in response to increases in the negative hydrostatic pressure applied to patch pipettes (27). These data suggest that \(\alpha\)-subunits may oligomerize to form channels that are mechanosensitive and participate in hepatocyte volume regulation.

Functional effect of coexpression of NH\(_2\)-terminal \(\alpha\)mENaC with wild-type Na\(^+\) channels. The putative cytoplasmic domains of each ENaC subunit comprise a small fraction of the total mass of the channel protein and contain regions critical for ENaC activity. For example, regulatory motifs in the COOH termini of \(\beta\) and \(\gamma\)ENaC including proline-rich domains and tyrosine-based internalization signals have been implicated in ENaC regulation and protein-protein interactions. Mutations or deletions of internalization signals or sites of interaction with Nedd4 have been associated with increases in functional ENaC activity (43, 46, 53, 54). A recent study suggested that the NH\(_2\) terminus of \(\gamma\)ENaC participates in subunit-subunit interactions and in subunit assembly (1). Studies of other ion channels, such as voltage-gated K\(^+\) channels, suggest that NH\(_2\) termini are important sites of subunit-subunit interaction (56). We examined whether the NH\(_2\) terminus of \(\alpha\)mENaC might be a site of subunit-subunit interactions. Our data suggest that the NH\(_2\) terminus of \(\alpha\)mENaC may serve as an intersubunit association domain, as coexpression of the NH\(_2\) terminus of \(\alpha\)mENaC with wild-type \(\alpha\), \(\beta\), and \(\gamma\)mENaC subunits in Xenopus oocytes inhibited amiloride-sensitive current. The mechanisms of suppression of ENaC expression are unclear, but the data are consistent with a disruption in normal assembly by formation of a heterologomer complex between the NH\(_2\) terminus of \(\alpha\)mENaC and wild-type \(\alpha\), \(\beta\), and/or \(\gamma\)mENaC subunits.

Type 1 pseudohypoaldosteronism (PHA1) is a disorder exhibiting Mendelian inheritance that is characterized by salt wasting, metabolic acidosis, and hyperkalemia. Our data suggest that the NH\(_2\)-terminal domain of \(\alpha\)ENaC functions as a dominant negative mutant when coexpressed with wild-type subunits, perhaps by inhibiting assembly of functional Na\(^+\) channels. Our dose-response experiments highlight the fact that inhibition of ENaC function was most profound when an excess of NH\(_2\)-terminal \(\alpha\)mENaC was expressed with wild-type \(\alpha\)mENaC (Fig. 7). Genotyping and single-strand chain polymorphism analysis of several autosomal recessive PHA1 kindreds have identified frame-shift mutations within human \(\alpha\) and \(\beta\)ENaC prior to the first trans-
and in association with increased potassium intake.

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Address for reprint requests and other correspondence: Y. J. Ahn, Renal Electrolyte Hypertension Division, Univ. of Pennsylvania, 700 Clinical Research Bldg., 422 Curie Boulevard, Philadelphia, PA 19104-6144 (E-mail: yahn@mail.med.upenn.edu).

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