Gating Induces a Conformational Change in the Outer Vestibule of ENaC

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Abstract. The epithelial Na⁺ channel (ENaC) is comprised of three homologous subunits (α, β, and γ). The channel forms the pathway for Na⁺ absorption in the kidney, and mutations cause disorders of Na⁺ homeostasis. However, little is known about the mechanisms that control the gating of ENaC. We investigated the gating mechanism by introducing bulky side chains at a position adjacent to the extracellular end of the second membrane spanning segment (549, 520, and 529 in α, β, and γENaC, respectively). Equivalent “DEG” mutations in related DEG/ENaC channels in Caenorhabditis elegans cause swelling neurodegeneration, presumably by increasing channel activity. We found that the Na⁺ current was increased by mutagenesis or chemical modification of this residue and adjacent residues in α, β, and γENaC. This resulted from a change in the gating of ENaC; modification of a cysteine at position 520 in βENaC increased the open state probability from 0.12 to 0.96. Accessibility to this side chain from the extracellular side was state-dependent; modification occurred only when the channel was in the open conformation. Single-channel conductance decreased when the side chain contained a positive, but not a negative charge. However, alterations in the side chain did not alter the selectivity of ENaC. This is consistent with a location for the DEG residue in the outer vestibule of ENaC. Disruption of this mechanism could be important clinically since one of the mutations that increased Na⁺ current (γN390K) was identified in a patient with renal disease.

Key words: hypertension • amiloride • sodium channel • epithelia • degenerin

Introduction

The epithelial Na⁺ channel (ENaC) is expressed at the apical membrane of epithelia, where it functions in Na⁺ absorption (Benos et al., 1995; Garty and Palmer, 1997). The channel is a heteromeric complex of α, β, and γENaC subunits (Canessa et al., 1994; McDonald et al., 1995). In the kidney collecting duct, ENaC plays a critical role in Na⁺ homeostasis and blood pressure control; mutations cause inherited forms of hypertension (Liddle’s syndrome) and hypotension (pseudohypoaldosteronism type 1; Snyder et al., 1995; Lifton, 1996). In the lung and intestine, ENaC controls the quantity and electrolyte composition of the surface liquid (Hummler et al., 1996; Zabner et al., 1998). In cystic fibrosis, defective regulation of ENaC may contribute to the pathogenesis of airway disease (Boucher et al., 1986).

Little is known about the mechanisms that control the gating of ENaC. In contrast to voltage- and ligand-gated ion channels, ENaC conducts current in the absence of an identifiable stimulus (Canessa et al., 1994; Benos et al., 1995; McDonald et al., 1995; Garty and Palmer, 1997). However, at the single-channel level, there is wide variability in the gating of the channel (Schild et al., 1995; Snyder et al., 1995). A bimodal Pₒ distribution also has been suggested, with channels existing in either a high or low Pₒ mode (Palmer and Frindt, 1996). Sequences in the cytoplasmic NH2 terminus may be involved in the gating of ENaC; mutations in this segment disrupted the ability of the channel to open (Grunder et al., 1997, 1999), and a loss-of-function mutation associated with pseudohypoaldosteronism type 1 was located in this segment (Grunder et al., 1997). It was also reported that the subunit composition could alter gating; channels comprised of only α and β subunits (without γ) had a very high Pₒ, whereas channels derived from coexpression of α and γ existed in either a high or low Pₒ state (Fyfe and Canessa, 1998). However, in epithelia, all three subunits appear to be required to generate Na⁺ current (Ishikawa et al., 1998; Snyder, 2000).

To investigate the gating mechanisms of ENaC, we examined the effect of mutations in amino acids near the extracellular end of the second membrane-spanning segment. Several findings suggest that this domain might be involved in channel gating. First, in related Caenorhabditis elegans (C. elegans) channels (MEC-4, MEC-10, and DEG-1), dominant gain-of-function mutations in this domain cause neuronal swelling and de-
generation, presumptively by increasing channel activity (Tavernarakis and Driscoll, 1997). These DEG mutations change an alanine to amino acids with a bulky or charged side chain. Second, mutation of a glycine at the equivalent position altered the function of ENaC (ASIC2), a mammalian member of the DEG/ENaC family expressed in neurons (Price et al., 1996). Mutation of this glycine to bulky or charged amino acids converted ENaC from a proton-activated channel to a channel that was active at neutral pH (Waldmann et al., 1996; Adams et al., 1998). Finally, in a previous study, we found that covalent modification of cysteines introduced in this domain in γENaC increase Na+ current (Snyder et al., 1999). In each ENaC subunit, a serine is located at the position equivalent to the C. elegans DEG residue (see Fig. 1). Interestingly, mutation of the DEG residue in MEC-4 to serine did not result in neurodegeneration, suggesting that substitution of a serine at this position is a conservative change. In this work, we investigated the mechanism by which DEG mutations in ENaC increase current, and the location of this residue within the channel complex.

MATERIALS AND METHODS

DNA Constructs

cDNA constructs for human α, β, and γENaC in pMT3, pcDNA3 (McDonald et al., 1994, 1995), or pGEM-HE (Volk et al., 2000) were generated as previously described, mutated using the QuickChange kit (Stratagene), and sequenced in the University of Iowa DNA Core. cRNA was transcribed using the Message Machine kit (Ambion).

Expression and Whole-Cell Electrophysiology

For measurement of whole-cell current, cDNAs encoding α, β, and γENaC (0.2 ng each; in pMT3 or pcDNA3) were injected into the nucleus of Xenopus oocytes. After incubation in modified Barth’s solution at 18°C for 16–24 h, we measured whole-cell Na+ currents by two-electrode voltage clamp with the cells bathed in 116 mM NaCl, 2 mM KCl, 0.4 mM CaCl₂, 1 mM MgCl₂, and 5 mM Hepes, pH 7.4, with NaOH. Amiloride-sensitive current was determined at −60 mV by adding a maximal dose (100 μM) to the bathing solution. Current-voltage relationships were obtained by stepping from −60 mV to potentials between −120 and +40 mV (20-mV steps) for 300 ms, Permeability ratios were calculated from changes in reversal potential with Na+ (20-mV steps) for 300 ms. Permeability ratios were calculated from changes in reversal potential with Na+, Li+, and K+ as the predominant cation in the extracellular bathing solution (Hille, 1992).

The response to methanethiosulfonate (MTS) compounds was determined by addition to the bathing solution of 1 mM MTSET ([2-(trimethylammonium)ethyl]methanethiosulfonate bromide), 10 mM MTSES (sodium (2-sulfonatoethyl)MTS), or 1 mM MTSEAdiiodoacetic acid (N-biotinylcaptoprolymoethanol MTS; Toronto Research Chemicals). These compounds have no significant effect on wild-type ENaC currents (Snyder et al., 1999). The percent change in amiloride-sensitive Na+ current was calculated as [(I_{AMTS} – I_{basal})/I_{basal}] × 100, where I_{AMTS} is the amiloride-sensitive current after treatment with an MTS reagent, and I_{basal} is the amiloride-sensitive current before treatment.

Single-Channel Currents

α, β (wild-type or β_{S520C}), and γENaC were expressed in Xenopus oocytes by cytoplasmic injection of cRNA (2 ng each). 1–3 d after injection, single-channel currents were recorded from devitellinized oocytes by patch-clamp (cell attached configuration). The pipet solution contained 150 mM LiCl, 1 mM CaCl₂, 1 mM MgCl₂, 5 mM Hepes, pH 7.4, with LiOH. The bath solution contained 150 mM LiCl, 5 mM EDTA, and 5 mM Hepes, pH 7.4, with LiOH. Currents were amplified using an Axopatch 200B amplifier (Axon Instruments) and acquired at 2 kHz using Pulse software (version 8.09; HEKA). Currents were digitally filtered at 100 Hz and analyzed using TAC 3.0 (Buxton Corporation). Slope conductance was determined between −100 and −40 mV (the conductance was identical in recordings filtered at 100 or 1,000 Hz). Open state probability (P_o) was determined at −100 mV in patches containing one to three channels. The majority of patches contained a single channel. Mean open and closed times were determined from patches containing single channels.

To selectively modify channels in the patch, 1 mM MTSET or 10 mM MTSES were included in the patch pipet. In some experiments, we used a lower concentration of MTSET (10 μM), and filled the tip of the pipet with solution lacking MTSET, to allow us to record currents before and after modification of the channel.

RESULTS

Bulky DEG Residue Side Chains in βENaC Increase Na+ Current

To test the hypothesis that mutations of the DEG residue in ENaC increase Na+ current, we mutated Ser²⁰⁰ in the β subunit (Fig. 1) to amino acids with larger and/or charged side chains. Expression of β_{S520R} (with wild-type α and γENaC) in Xenopus oocytes generated 3.2-fold more amiloride-sensitive Na+ current than wild-type ENaC (Fig. 2, A and B). This mutation increased the size of the side chain at the DEG position, and added a positive charge. Mutation of Ser²⁰⁰ to an amino acid with a large neutral side chain (β_{S520K}) or a negatively charged side chain (β_{S520E}) also increased Na+ current (Fig. 2 B). In contrast, a more conservative mutation that did not change the size or charge of the side chain (β_{S520C}) did not increase Na+ current (Fig. 2 B).

Because the β_{S520C} mutation did not alter Na+ current, this allowed us to acutely change the composition of the DEG residue side chain by covalent modification of the cysteine. MTSET attaches an (S)-ethyl trimethyl ammonium group to the cysteine, increasing the size and adding a positive charge to the side chain. When we expressed β_{S520C} (with wild-type α and γENaC), extracellular MTSET rapidly increased Na+ current 4.3-

\[ \text{DEG Residue} \]
\[ \alpha \text{ ENaC} \quad 543 \text{TMVTLISNLGSQSWLFGSSLVSVE} \ldots \]
\[ \beta \text{ ENaC} \quad 514 \text{NIVLWISNLGGQGFWMGSVLCLIE} \ldots \]
\[ \gamma \text{ ENaC} \quad 523 \text{SIEMLISNFQGQLWMSCSIWCVIE} \ldots \]
\[ \text{MCE-4} \quad 707 \text{GVNLADFGQLGLWCGTIFLTCE} \ldots \]
\[ \text{BNC1} \quad 824 \text{EAVALGIDQCGMFLIGASLITLE} \ldots \]

Figure 1. Pre-M2 segment of DEG/ENaC ion channels. DEG residue is indicated by a black box; and the shaded box indicates ENaC residues that form selectivity filter. Numbers indicate the first amino acid for each subunit.
fold (Fig. 3, A and B). Amiloride completely blocked the stimulated current, indicating that it resulted from the stimulation of ENaC. Also consistent with this interpretation, MTSET had no significant effect on uninjected oocytes (not shown) or oocytes expressing wild-type ENaC (Snyder et al., 1999). MTSET-induced stimulation was irreversible upon removal of MTSET from the bathing solution, but was reversed by the addition of a reducing agent, dithiothreitol (20 mM), to the bathing solution (Fig. 3 A). These results, together with the lack of effect of MTSET on wild-type ENaC, indicate that stimulation resulted from covalent modification of the cysteine introduced at the DEG position.

To determine whether the size or the charge of the side chain (or both) was required for MTSET-induced stimulation of ENaC, we tested the effect of different MTS compounds. MTSES is similar in size to MTSET, but it carries a negative charge. Modification of $\beta_{SS20C}$ with MTSES increased Na$^+$ current to the same extent as MTSET (Fig. 3 B). A large neutral compound (MT-SEA-biotincap) also increased Na$^+$ current (Fig. 3 B). These results, together with our finding that large neutral, positive, and negative amino acids increased Na$^+$ current (Fig. 2 B), suggest that the size of the DEG residue side chain, rather than its charge, was responsible for the stimulation of ENaC.

**DEG Mutations in $\alpha$ and $\gamma$ENaC**

The three ENaC subunits share significant sequence similarity, including the serine at the DEG position in all three subunits (Fig. 1). Therefore, we tested the hypothesis that an increase in the size of the side chain at the DEG position in $\alpha$ and $\gamma$ENaC would increase Na$^+$ current as it did in $\beta$ENaC. When we placed a cysteine at the DEG position in $\alpha$ ($\alpha_{SS49C}$) or $\gamma$ENaC ($\gamma_{SS29C}$) (co-expressed with the other two wild-type subunits), Na$^+$ currents were identical to wild-type ENaC (Fig. 4 C). Modification of the cysteine introduced in $\alpha_{SS49C}$ with MTSET increased Na$^+$ current (Fig. 4 A), although to a lesser extent than modification of $\beta_{SS20C}$ (Fig. 4 B). In contrast, modification of $\gamma_{SS29C}$ had minimal effect on Na$^+$ current (Fig. 4, A and B; Snyder et al., 1999); either the DEG residue in $\gamma$ENaC was not accessible to modification, or modification did not alter the current. In addition, mutation of $\gamma_{SS29}$ to either valine or lysine also failed to increase the current (Fig. 4 C), suggesting that, in $\gamma$ENaC, large side chains at the DEG position do not alter channel function. Conversely, Na$^+$ current decreased when we mutated the DEG residue (Ser$^{549}$) to valine or lysine in $\alpha$ENaC (Fig. 4 C). This contrasts with the increase in current when $\alpha_{SS49C}$ was modified by MTSET (Fig. 4 A).
We asked whether bulky side chains at positions surrounding the DEG residue would also stimulate the channel. When a cysteine was introduced at position 521 in βENaC, MTSET increased Na⁺ current (Fig. 4B). Stimulation was identical when cysteines were modified at the equivalent position in α or γENaC (positions 550 and 530, respectively; Fig. 4B). In contrast, modification of a cysteine introduced at the other neighboring position in α and βENaC (positions 548 and 519, respectively) did not increase Na⁺ current. However, in γENaC, modification of this cysteine (position 528) produced a large increase in Na⁺ current (Fig. 4B). Thus, stimulation was not specific to the DEG position, but resulted from introduction of a bulky side chain at neighboring positions as well. The data suggest that the DEG region has a similar function in all three ENaC subunits, although there are differences in the function of the specific residues.

A sequence variation in this DEG domain was identified in a patient with diabetic nephropathy, changing Asn530 in γENaC to lysine (γN530K; Melander et al., 1998). This change increases the size of the side chain and introduces a positive charge, similar to modification with MTSET. We found that expression of γN530K (with wild-type α and βENaC) increased Na⁺ current twofold compared with wild-type ENaC (Fig. 4D). Thus, a mutation in the DEG domain might alter the function of ENaC in humans.

Effect of Cysteine Modification on Selectivity

ENaC is highly selective for Na⁺ over K⁺, and is slightly more permeable to Li⁺ than Na⁺ (Benos et al., 1995; Garty and Palmer, 1997). This is illustrated by the current-voltage relationships for wild-type ENaC with Na⁺, Li⁺, or K⁺ as the predominant cation in the extracellular bathing solution (Fig. 5A). To test the hypothesis...
that the DEG residue contributes to the selectivity properties of ENaC, we determined the cation permeability ratios for mutant and chemically modified channels. Mutation of Ser1920 in βENaC to lysine or cysteine did not alter either the Na+/Li+ or K+/Na+ permeability ratios (Fig. 5, B and C, respectively). Modification of a cysteine at this position with either MTSET or MTSES also did not significantly alter selectivity (Fig. 5, B and C). As positive controls, mutation of two adjacent residues in the second membrane-spanning segment (γS540C and γS542C; Fig. 1, shaded box) changed the selectivity of ENaC (Fig. 5, B and C), similar to previous reports (Kellenberger et al., 1999a,b; Snyder et al., 1999). These results suggest that the DEG residue does not contribute to the selectivity filter of ENaC.

**MTSET Locks βS520C in a High Po State**

The presence of a bulky side chain at the DEG position could stimulate ENaC by increasing either the single-channel conductance or the Po. To examine these mechanisms, we measured single-channel currents in cell-attached patches of cells expressing βS520C with wild-type α and γENaC. To modify the DEG cysteine, MTSET was included in the patch pipet in an independent group of patches. Fig. 6 A shows representative currents from patches containing single channels. Without MTSET, βS520C displayed kinetics similar to previous reports for wild-type ENaC (Fig. 6 A, top; Palmer and Frindt, 1986; Canessa et al., 1994; Snyder et al., 1995; Volk et al., 1995). However, the Po (0.12 ± 0.03; Fig. 6 B) was lower and less variable (Po = 0.005–0.28) than reported for wild-type human ENaC (0.58 ± 0.12; Snyder et al., 1995). MTSET caused a dramatic change in kinetics, converting the βS520C channel from a low to a high Po state (0.96 ± 0.03; Fig. 6, A and B). As illustrated in the single-channel trace (Fig. 6 A), the channel was almost always open, interrupted by only brief closures. Increased Po resulted from both an increase in the mean open time (Fig. 6 D) and a decrease in mean closed time (Fig. 6 E). MTSET had no effect on the gating of wild-type ENaC (not shown). βS520K increased whole-cell Na+ current in the absence of MTSET (Fig. 2 B). This also resulted from an increase in Po (0.96 ± 0.01, n = 4), similar to the modification of βS520C with MTSET.

In the absence of MTSET, βS520C had a single-channel conductance (8.4 pS for Li+) similar to wild-type human ENaC (Fig. 6 C; Snyder et al., 1995). Surprisingly, MTSET decreased the single-channel conductance to 5.8 pS. Thus, an increase in conductance did not contribute to the MTSET-mediated stimulation of ENaC. However, the negatively charged MTSES did not decrease the single-channel conductance (Fig. 6 C), suggesting that the positive charge of MTSET was required. MTSET did not alter the single-channel conductance of wild-type ENaC (not shown).

**Modification of βS520C Is State Dependent**

To investigate the conformational changes associated with the gating of ENaC, we tested the hypothesis that βS520C was modified selectively in either the open or closed conformation. Modification of the channel by MTSET changes the gating and single-channel conductance, converting ENaC from a low Po, large single-channel conductance (O_L) state to a high Po, small single-channel conductance (O_S) state. If βS520C was modified in the closed conformation, we predict that the next channel opening would be to the O_S/high Po state. This concept is illustrated in Fig. 7 A (top). In contrast, if modification occurred when the channel was open, we predict that the channel would first open into the O_L state, followed by a decrease in current to the O_L/high Po state at the time of modification (Fig. 7 A, bottom). In the protocol used in Fig. 6, the rate of modification was too fast to allow us to record channel activity at the time of modification. To delay modification, we used a lower concentration of MTSET (10 μM) in the patch pipet, and filled the tip of the pipet with
solution lacking MTSET. Using this approach, we were able to observe the transition from the O_1/low P_o to the O_3/high P_o state in 10 experiments. An example is shown in Fig. 7 B. The patch contained a single channel that opened only to the O_1 state during the first 4.6 min of recording (the last 8.5 s are shown, Before Modification). The P_o during this time was very low (0.01). The channel then converted (Fig. 7 B, Modification) to the O_3/high P_o state for the remainder of recording. After modification, the channel had brief infrequent closures (Fig. 7 B, inset c) with a P_o close to 1.0, and there was a significant decrease in the single-channel current amplitude (Fig. 7 D), which is consistent with conversion from the O_1 to the O_3 state. In Fig. 7 B, inset b shows an expanded time scale to focus on this conversion between states. The channel first opened into the O_1 state, followed by a decrease in current to the O_3 state (indicated by the arrowhead). This sequence is consistent with modification of the channel in the open conformation; it was observed in 10/10 experiments (Fig. 7 A). A second example is shown in Fig. 7 C. The three sweeps were taken from the same channel before, during, and after modification with MTSET. This record contains longer closures from the O_3 state. We did not observe channels open directly into the O_3 state (Fig. 7 A). Thus, the data suggest that modification of the DEG residue is state-dependent, occurring selectively in the open conformation.

**DISCUSSION**

Our data indicate that the DEG residue, and adjacent residues, are involved in the gating of ENaC. Large side chains at this position disrupt a conformational change in the outer vestibule, locking the channel in a high P_o state.

Several findings suggest that the DEG residue influences the conduction pathway of ENaC. First, a cysteine introduced at this position was accessible to modification with water-soluble thiol-reactive compounds added to the extracellular bathing solution. Second, modification of β_520C with MTSET decreased the single-channel conductance. Third, a positive charge was required; the negatively charged MTSES did not decrease single-channel conductance. This electrostatic effect suggests that the DEG residue is located within the conduction pathway, and is reminiscent of the effect of charged residues in the outer vestibule on the conductance of the nicotinic acetylcholine receptor (Imoto et al., 1988). Also consistent with this model is the previous report that a mutation at the DEG position decreased the ability of a cation (MTSET) to enter the pore and modify a cysteine in ENaC (Eskandari et al., 1999). However, the DEG residue does not appear to be part of the selectivity filter since modification or mutation of this residue did not alter the selectivity of ENaC (Li et al., 2007). Thus, the data are consistent with a model in which the DEG residue is located in the outer vestibule of the channel. This also fits with our previous finding that, in γENaC, residues in the DEG domain lie external to the site in the pore of amiloride block (Snyder et al., 1999).

The presence of a bulky side chain at the DEG position produced a dramatic change in the gating of ENaC, converting the channel from a low P_o state to a...
state in which the channel was almost always open. This resulted from a destabilization of the closed state and stabilization of the open state, as reflected by the large decrease in closed time and increase in open time, respectively. The requirement for a bulky side chain suggests that this may be a steric effect; perhaps the bulky side chain interferes with the conformational change required for the channel to close. Such a mechanism was previously proposed to explain the swelling neurodegeneration produced by equivalent mutations in *C. elegans* (Driscoll and Chalfie, 1991). It seems likely that modification of cysteines at the DEG position and surrounding positions in α and γENaC increased Na⁺ current by increasing Pₒ, similar to the modification of βS20C. However, we cannot exclude an increase in the single-channel conductance of these mutant channels.

We used two strategies to alter the side chain at the DEG position: mutagenesis to amino acids with large and/or charged side chains, and modification of an introduced cysteine. Both strategies produced equivalent results, with one exception; modification of αS549C increased Na⁺ current, whereas mutation of this residue to valine or lysine decreased current. The reason for this difference is unclear. It is possible that large side chains at the DEG position in αENaC disrupted the processing of ENaC to the cell surface, resulting in decreased Na⁺ current. This underscores a significant advantage of the cysteine modification strategy, which allowed us to determine the functional effect of an acute change in the size and/or charge of the side chain. A second possible mechanism involves the number of altered αS549 side chains in the channel complex. ENaC
contains either two or three α subunits (Firsov et al., 1998; Kosari et al., 1998; Snyder et al., 1998; Eskandari et al., 1999). However, we don’t know how many of the α subunits were modified by MTSET; modification of only one cysteine might be sufficient to increase current. In contrast, when we mutated αS549 to valine or lysine, each α subunit contained a large side chain. Perhaps one large α side chain increases current, but current is decreased when all of the α subunits contain large side chains. Finally, the modified cysteine is not identical to lysine or valine; differences in the structure of the side chain could also explain the data. Future work will be required to distinguish between these potential mechanisms. The increase in whole-cell Na⁺ current with modification of βS520C by MTSET (3.3–4.2 fold; Figs. 3 B and 4 B) was less than predicted (5.4-fold increase) from the increase in Po (8-fold) and decrease in single-channel current (0.67-fold). It is possible that some channels or DEG cysteines may not have been modified by MTSET.

We found that a cysteine introduced at the DEG position was modified only when the channel was in the open conformation. This state-dependent modification suggests that channel gating results from a conformational change that alters the accessibility of the DEG residue to the extracellular bathing solution. Two potential models could explain these results. First, channel gating might result from the opening and closing of a gate in the extracellular domain of ENaC (Fig. 8, top). If the gate was external to the DEG residue, channel closure would block access to this residue. Steric hindrance by the bulky side chain at this position might make it unfavorable for the gate to close. In this model, channel gating does not change the position of the DEG residue in relation to the pore of ENaC. This contrasts with a second potential model in which the DEG residue changes position during the gating conformational change (Fig. 8, bottom). When the channel is open, the DEG residue side chain lines the vestibule where it is accessible to modification. Channel closure moves the DEG residue into a buried inaccessible position. A bulky side chain at the DEG position prevents this conformational change, disrupting channel closing. In this model, the gate lies internal to the DEG residue, either at the selectivity filter or within the intracellular vestibule, similar to K⁺ channels. The two models shown in Fig. 8 are not mutually exclusive: elements of both models could be true. However, common to both models is the requirement for a conformational change in the outer vestibule in the gating of ENaC.

There are fundamental differences between the gating of ENaC and other members of the DEG/ENaC family of Na⁺ channels. Whereas nearly all of these channels require a ligand for the channel to open, ENaC is active in the absence of any known stimulus. However, there are also similarities. A bulky side chain at the DEG position increases the activity of several members of the DEG/ENaC family. In BNC1, the accessibility of a cysteine at the DEG position changed in response to acidic pH; this cysteine was relatively inaccessible at neutral pH, but became accessible at pH 5 (Adams et al., 1998). Thus, the mechanism that underlies the gating of ENaC may be a conserved feature of the DEG/ENaC family.

DEG mutations produce pathology. In C. elegans, DEG mutations in MEC-4, MEC-10, and DEG-1 cause neuronal swelling, lysis, and touch insensitivity. In ENaC, a DEG mutation may also have clinical rele-
vance. Melander and co-workers identified a patient with diabetic nephropathy who had a mutation in the γ subunit, changing Asn530 to lysine (Melander et al., 1998). Interestingly, we found that this mutation increased Na⁺ current. Modification of a cysteine at this position with MTSET also increased Na⁺ current. Thus, its seems possible that mutation of Asn530 might predispose to hypertension, and be a contributing factor to renal disease in this patient. Further work will be required to determine the frequency of this mutation in the population and its role in disease.

We thank Tien Vinh, Sarah Hestekin, and Fotene Gennatos for technical support and Michael Welsh, Christopher Adams, Christopher Benson, and our other laboratory colleagues for helpful discussions and critical review of this manuscript. We thank Ken Volk and John Stokes for providing ENaC subunits in pGEM-HE.

P.M. Snyder was supported by the Roy J. Carver Charitable Trust and by the National Heart, Lung and Blood Institute (grants No. HL-58812 and HL-03575) and National Institute of Diabetes and Digestive Kidney Diseases (grant No. DK-52617) of the National Institutes of Health.

Submitted: 11 May 2000
Revised: 13 October 2000
Accepted: 16 October 2000

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