Mutations Causing Neurodegeneration in Caenorhabditis elegans Drastically Alter the pH Sensitivity and Inactivation of the Mammalian H⁺-gated Na⁺ Channel MDEG1*

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Guy Champigny, Nicolas Voilley, Rainer Waldmann, and Michel Lazdunski‡†

From the Institut de Pharmacologie Moleculaire et Cellulaire, CNRS-UPR 411, Sophia Antipolis, 06560 Valbonne, France

The mammalian degenerin MDEG1 belongs to the nematode degenerin/epithelial Na⁺ channel superfamily. It is constitutively activated by the same mutations that cause gain-of-function of the Caenorhabditis elegans degenerins and neurodegeneration. ASIC and DRASIC, which were recently cloned, are structural homologues of MDEG1 and behave as H⁺-gated cation channels. MDEG1 is also a H⁺-activated Na⁺ channel, but it differs from ASIC in its lower pH sensitivity and slower kinetics. In addition to the generation of a constitutive current, mutations in MDEG1 also alter the properties of the H⁺-gated current. Replacement of Gly-430 in MDEG1 by bulky amino acids, such as Val, Phe, or Thr, drastically increases the H⁺ sensitivity of the channel (half-maximal pH (pH₅₀) ≈ 4.4 for MDEG1, pH₅₀ ≈ 6.7 for the different mutants). Furthermore, these replacements completely suppress the inactivation observed with the wild-type channel and increase the sensitivity of the H⁺-gated channel to blockade by amiloride by a factor of 10 without modification of its conductance and ionic selectivity. These results as well as those obtained with other mutants clearly indicate that the region surrounding Gly-430, situated just before the second transmembrane segment, is essential for pH sensitivity and gating.

Hereditary neurodegeneration in Caenorhabditis elegans is caused by mutations of the degenerins DEG-1, MEC-4, and MEC-10 (1–3). We previously cloned a new member of the epithelial Na⁺ channel/degenerins superfamily (1–12) MDEG1 (or BNaC1) from human and rat brain, which behaves like the nematode degenerins (13–15). At physiological pH, MDEG1 lacked channel activity when expressed in Xenopus oocytes. However, the same mutations that induce neurodegeneration in C. elegans degenerins can transform MDEG1 into an amiloride-sensitive cation channel with a low conductance (2–4 pS) and a low Na⁺ selectivity (PNa+/PK⁺ = 3–5) (13). This constitutive activation of MDEG1, through very selective mutations, leads to the swelling and death of cells which express mutated MDEG1 (13). These mutations correspond to the replacement of the Gly-430 residue situated just before the second transmembrane domain of MDEG1, by bulky amino acids such as Val, Phe, or Thr. These results suggested that the Gly-430 residue of the channel MDEG1 was part of an inhibitory domain of a channel normally activated by an unknown ligand.

More recently, MDEG1 was found to be activated by rapid external acidifications and to belong to the newly identified family of neuronal H⁺-gated channels (16). This channel family is actually composed of ASIC (or BNaC2) (15), a fast inactivating H⁺-gated Na⁺ channel (17), DRASIC, a non-inactivating Na⁺ channel, which probably plays an important role in nociception (18), and MDEG2 a splice variant of MDEG1, which is not active by itself but which associates with both MDEG1 and DRASIC to modulate their kinetics and pH sensitivity (16). MDEG1 differs from ASIC, the first identified member of the H⁺-gated channel family, in its sensitivity to more acidic pH and by its slower kinetics (16). MDEG1 can associate with ASIC to form a heteromer with new properties (19).

The present study extends the characterization of the properties of the H⁺-gated channel MDEG1 and uses the Xenopus oocyte expression system to show that the same mutations, which in C. elegans degenerins cause neurodegeneration, drastically alter both the pH sensitivity and inactivation process of the mammalian H⁺-gated channel MDEG1. This work clearly identifies a specific region situated just before the second transmembrane domain as playing a crucial role in both pH sensing and control of channel kinetics.

EXPERIMENTAL PROCEDURES

Construction of Expression Vectors and Mutagenesis—The wild-type and mutant MDEG1 cDNAs were inserted into the pBSK-SP6-globin vector as described previously (13).

Expression in Oocytes and Electrophysiological Analysis—For expression in Xenopus oocytes, cRNA was synthesized from the NotI-digested vector using a kit from Stratagene. Xenopus oocytes were injected with 0.05–5 ng of cRNA, and microelectrode voltage-clamp and patch-clamp recordings were performed 1–3 days after injection. For MDEG1 mutants, which induced a large constitutive current (13), oocytes were kept in low Na⁺ medium containing 10 mM NaCl and 86 mM NMDGCl. For outside-out patches the pipettes contained 140 mM KCl, 2 mM MgCl₂, 5 mM EGTA, 10 mM HEPES, pH 7.4 (with KOH). The bath medium contained 140 mM NaCl, LiCl, or N-methyl-D-glucamine chloride, 2 mM MgCl₂, 1.8 mM CaCl₂, 10 mM HEPES, pH 7.4 (adjusted with HCl, NaOH, LiOH, or tetramethylammonium chloride). Data were sampled at 2 kHz and filtered at 500 Hz for analysis (Biopatch software). The fast change of bathing solutions was accomplished by a rapid solution changer (RSC200, Biologic). Time of rotation from one tube to the adjacent was fixed at 5 ms. Intracellular acidification of oocytes was obtained by injection of 50 nl of the internal solution at pH 2 or by perfusion and removal of a bath medium containing 20 mM NH₄Cl.

RESULTS AND DISCUSSION

We previously reported that MDEG1 expressed in Xenopus laevis oocytes could not be activated by pH changes from the
physiological external pH of 7.4 to pH 6, i.e., by conditions which fully activate ASIC (17). However, a more extensive investigation revealed that MDEG1, when expressed in oocytes, can in fact generate a rapidly activating and inactivating inward current in response to pH pulses to below pH 5.5 (Fig. 1A). Very significant channel activations occur for pH jumps from pH 7.4 to pH values below 5. The half-maximal value of this activation by pH is pH50 = 4.35 (Fig. 1A). The H+ directly activate MDEG1 since H+-gated channels can be recorded in cell free membrane outside-out patches (Fig. 1A). While extracellular acidification clearly activates the MDEG1 channel, intracellular acidification of oocytes failed to activate MDEG1 at an external pH of 7.4, nor did it alter the MDEG1 current induced by extracellular H+ after a pH jump from 7.4 to 4 (not shown). As it was previously observed in central neurons (20) the inactivation process of the H+-gated channel can be described by a single exponential function. The rate of inactivation increases as the external pH is made more acidic (τpH5.5 = 1.65 ± 0.2 s, τpH3.5 = 0.129 ± 0.017 s (n = 15)) but does not show any significant voltage dependence (not shown).

The H+-gated current generated by the MDEG1 channel is blocked by amiloride with an IC50 value of 28 μM (Fig. 1B), is more permeable to Na+ and Li+ as compared with K+ (PNa+/PK+ = 10), and is not significantly permeable to Ca2+ (Fig. 1C). Na+ can thus be considered as the major permeant ion under physiological conditions. The single channel conductance is 10.4 pS (Fig. 1D).

MDEG1 is expressed in neurons in many different regions of the brain (13, 15, 16) such as hippocampus, cerebellum, and cortex. Several types of H+-activated channels have been recorded in sensory and in hypothalamic neurons, but none has the properties which are described here for MDEG1 (21). It is intriguing that the pH dependence of the channel occurs in such a low pH range. Extracellular pH variations have been observed in the brain, which have led to the suggestion that the H+ could have a neurotransmitter role (22, 23). The existence in the brain of H+-gated channels like ASIC, which are activated at pH values below 7, would tend to support this view (17). However, one does not really know whether neuronal activity would produce large pH variations to below pH 5.5 at the synaptic level. MDEG1 channels can probably be activated in pathophysiological conditions such as ischemia and epileptic seizures, which produce large acidifications of the extracellular medium (24). Of course another possibility is that MDEG1 can associate with other subunits that change its pH dependence toward a more alkaline pH range. Indeed, MDEG1 can associate with ASIC, but the pH dependence of the MDEG1/ASIC channel remains fairly acidic (pH50 = 4.8) (19). The pH sensitivity of MDEG1 could also be modified by phosphorylation, by interactions with G proteins, or by a co-ligand, like the NMDA-gated ionic channel, which requires glycine to increase its glutamate sensitivity (25). Another possibility is that the pH sensitivity of MDEG1 might be a general feature of this new channel family and that MDEG1 is normally activated by a ligand other than H+.

Neurodegeneration in C. elegans is caused by mutation of an Ala residue in DEG-1 and MEC-4 degenerins, just before the second transmembrane domain (1, 3). The corresponding residue in MDEG1 is Gly-430. We previously demonstrated that mutations of Gly-430 caused a constitutive activation of MDEG1 (13). Properties of the H+-activated channel generated by MDEG1 at acidic pH are clearly distinct from those of the constitutively active mutated channels at pH 7.4 (13). Gain-of-function mutant channels are more sensitive to amiloride (IC50 ~2–13 μM), less selective for cations (PNa+/PK+ ~3–5), less conductive (gNa+ ~2–4 pS), and more voltage-dependent than the H+-gated channel (PF ~0.5 at −40 mV and 0.1 at −120 mV).

Figs. 2 and 3 show that substitution of Gly-430 by amino acids that cause neurodegeneration in C. elegans degenerin MEC-4 (2) and constitutively activate MDEG1 at pH 7.4 also markedly alter the properties of the H+-gated current. The pH dependence of their channel activity was shifted to much less acidic pH values than for wild-type MDEG1. A shift of at least two pH units toward alkaline pH was observed for MDEG1-G430V (pH50 = 6.5) for MDEG1-G430T (pH50 = 6.7) and for MDEG1-G430F (pH50 = 6.9) (Fig. 3). Another striking observation is that the inactivation process (that occurs after H+ activation of the MDEG1 channel) is completely abolished in G430T and G430F mutants and markedly slowed by the G430C mutation (Fig. 2, for the G430V mutant, see Figs. 4 and 5).

The substitution of Gly-430 by Ser in MDEG1 does not
cause constitutive channel activity (1) (Fig. 2A) and the corresponding mutation in MEC-4 does not cause neurodegeneration (2). However, this mutation causes a small shift in the external pH dependence of MDEG1 from a pH$_m$ of 4.35 (MDEG1) to a pH$_m$ of 4.8 (MDEG1-G430S) (Fig. 3). It also produces a slower inactivation ($t_{pH 5.4}$ = 5.4 s, $t_{pH 4.9}$ = 29.1 s ($n$ = 6), Fig. 2B).

Since substitutions of Gly-430 by Val, Phe, or Thr, which constitutively activate the channel, also induce a marked shift in the external pH dependence of the MDEG1 channel, it remained a possibility that the basal current observed with all of these mutants was due to a partial activation of the H$^+$-gated channel at pH 7.4. However, this is clearly not the case. Changing the initial pH from pH 7.4 to 8.5 did not change the intensity of the constitutive current of the MDEG1-G430V mutant (at pH 7.4 and 8.5 $I_{270 mV}$ = 1.36 ± 0.06 ($n$ = 8)).

The single channel properties of different mutants of MDEG1 at acidic pH values are described in Fig. 4. During a pulse to pH 5 the mutant H$^+$-activated channels MDEG1-G430T and MDEG1-G430V remained permanently open until the pH returned to 7.4. Changes in the unitary current levels during the acidic step at pH 5 could be seen immediately after the removal of amiloride or when low concentrations of amiloride (1 μM), which are insufficient for complete blockade, were added (Fig. 4, A and B). Gly-430 mutations did not alter ionic selectivity and conductance of the H$^+$-gated channels (Figs. 1, C and D, and 4, D and E). The Na$^+$ versus K$^+$ selectivities are nearly identical (wild type, $P_{Na^+}/P_{K^+}$ = 10; G430T, $P_{Na^+}/P_{K^+}$ = 8).

**Fig. 2.** A, histogram of the basal current measured at pH 7.4 and at −70 mV for MDEG1 and for Gly-430 mutants. Each cRNA was injected at the same concentration of 1 ng/μl (0.05 ng/oocyte). The inset shows the effect of an increase of the G430C cRNA concentrations on the amplitude of the constitutive current measured at −70 mV and at pH 7.4. Number of experiments are indicated in the figure. The indicated sequences correspond to the domain just preceding the MII segment and the MII domain of MEC-4 and MDEG1. The Ala-713 residue of MEC-4 and Gly-430 residue of MDEG1 are marked with an asterisk. OUT and IN indicate the extra- and intracellular side of the transmembrane domain. B, typical recordings illustrating the effect of substituting the Gly-430 residue in MDEG1 by various amino acids on the H$^+$-activated current. Currents were recorded at 0 mV from outside-out patches. Drops in pH are indicated above each trace. WT, wild type.

**Fig. 3.** pH dependence of the H$^+$-gated channel activity of the Gly-430 mutants compared with the pH dependence of wild-type (WT) MDEG1. Points represent mean values calculated from 3–11 oocytes. H$^+$-activated currents were measured as current changes induced by external pH jumps from 7.4.
Gly-430 Mutations Increase H⁺ Sensitivity

Fig. 4. A and B, effect of amiloride on the currents activated at pH 5 and recorded from outside-out patches at 0 mV for the G430T and G430V mutants. C, dose-response curves for the amiloride effect on the G430T and G430V mutants. Percent inhibition was calculated on the mean current recorded from outside-out patches at pH 5 and 0 mV in the Na⁺-containing medium. D, mean I-V relationships of the H⁺-activated currents recorded at pH 5 from outside-out patches and measured by voltage ramps from −100 to 100 mV with 140 mM Na⁺ (reversal potential = 57 mV, P_Na⁺/P_K⁺ = 10) or 0 mM Na⁺ + 1.8 mM Ca²⁺ (reversal potential = −60 mV, P_K⁺/P_Ca²⁺ = 0.5) in the external solution. The H⁺-activated current was obtained by subtracting the constitutive current recorded at pH 7.4 from the current recorded after an acidification to pH 5. E, Mean i-V relationships of the G430T and G430V H⁺-gated channels activated at pH 5 and of the constitutive G430V channel at pH 7.4 recorded from outside out patches in Na⁺-containing medium. Data were collected from four different patches. The inset shows typical recordings of a H⁺-gated mutant channel G430V recorded in the presence of 1 μM amiloride.

Fig. 5. Long lasting outside-out patch recording showing three G430V channels at pH 7.4 (3 pS) and during pH 5 pulse (11 pS).

G430V, P_{Na⁺}/P_{K⁺} = 7; Figs. 1D and 4E) as well as the single channel conductances (wild type, g_{Na⁺} = 10.4 pS; G430T, g_{Na⁺} = 8.2 pS; G430V, g_{Na⁺} = 11 pS; Figs. 1D and 4E). However, Gly-430 mutations significantly increase the amiloride sensitivity (Figs. 1B and 4C, IC_{50 (WT)} = 28 μM, IC_{50 (G430T)} = 4 μM, IC_{50 (G430V)} = 2 μM).

Fig. 5 clearly shows that the channel activity of the MDEG1-G430V mutant produced by an acidic step is different from the constitutive channel activity recorded at pH 7.4, which has been previously described (13). Currents recorded at pH 7.4 are due to the activity of three channels of 3 pS with a relatively low selectivity for Na⁺ (Figs. 4E and 5), while currents recorded at pH 5 are due to the activity of three highly Na⁺-selective H⁺-gated channels of 11 pS (Figs. 4E and 5).

Therefore, MDEG1 Gly-430 mutants may express two different types of channel activity: (i) a constitutive channel activity, which is recorded at pH 7.4 (13), and (ii) an altered H⁺-gated Na⁺ channel activity. Clearly these two types of activities are strongly correlated: (i) mutations such as G430T, G430V, and G430F, which induce large constitutive currents, also produce large changes in the pH dependence of the H⁺-gated current and suppress inactivation; (ii) mutation G430C that causes a low level of constitutive channel activity is accompanied by an intermediate change in the kinetics and the pH dependence of
the H$^+$-gated Na$^+$ channel; and (iii) a mutation such as G430S that leads to only a small change of the kinetics and of the pH dependence of the H$^+$-gated channel produces essentially no constitutive activity. Because both types of channel activity are strongly correlated and because the number of active channels observed on the same patch before and during acidification is identical (see Fig. 5), it seems unlikely that these two different types of channel activity are due to different forms of mutated channels. In that case, different numbers of active channels would be observed before and during acidification, and superimposition of two types of activity would be observed. In fact, the same mutated channel may exist in the two conductance states, and the external pH may be responsible for the observed mode changes in channel activity.

The present work provides interesting information about structural elements that are essential for the gating system of H$^+$-gated channels. It clearly indicates that the part of the channel that includes Gly-430 has a key role in controlling the pH dependence, the inactivation kinetics, and also the amiloride sensitivity of this channel, but has no role in the control of the ionic selectivity and conductance. Gly-430 is situated in a hydrophobic environment situated just before the second transmembrane domain (13). This part of the protein has also been suggested to have a key role in the gating system of the epithelial amiloride-sensitive Na$^+$ channel (26). The logical link between glycine substitution by bulkier amino acids in MDEG1 and the shift of the pH sensitivity of the H$^+$-gated Na$^+$ channel is that these amino acid replacements probably produce a conformational change leading to a large modification of the apparent pK of the ionizable group(s) that control(s) this pH dependence.

It is particularly striking that all mutations in MEC-4 or DEG-1 that provoke neurodegeneration in C. elegans also induce large changes in pH dependence and inactivation in the MDEG1 channel, that a mutation that provokes an intermediate phenotype in C. elegans also induces an intermediate phenotype (G430C) for the H$^+$-gated Na$^+$ channel, and also that a mutation that provokes no degeneration in C. elegans has hardly any effect (G430S) on the MDEG1 H$^+$-gated channel function. This may suggest a similar function for both types of proteins.

Because abnormal channel activation is known to be the main cause of several inherited neurodegenerative diseases in mice (27, 28) and because the codon of the Gly-430 residue in the human DNA sequence of MDEG1 is situated at an intron-exon junction (GenBank™ accession no. 003687), it might turn out in the future that mutations in human MDEG1 may be implicated in some rare human neurodegenerative disease.

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