A Modulatory Subunit of Acid Sensing Ion Channels in Brain and Dorsal Root Ganglion Cells*

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MDEG1 is a cation channel expressed in brain that belongs to the degenerin/epithelial Na⁺ channel superfamily. It is activated by the same mutations which cause neurodegeneration in Caenorhabditis elegans if present in the degenerins DEG-1, MEC-4, and MEC-10. MDEG1 shares 67% sequence identity with the recently cloned proton-gated cation channel ASIC (acid sensing ion channel), a new member of the family which is present in brain and in sensory neurons. We have now identified MDEG1 as a proton-gated channel with properties different from those of ASIC. MDEG1 requires more acidic pH values for activation and has slower inactivation kinetics. In addition, we have cloned from mouse and rat brain a splice variant form of the MDEG1 channel which differs in the first 236 amino acids, including the first transmembrane region. This new membrane protein, which has been called MDEG2, is expressed in both brain and sensory neurons. MDEG2 is activated neither by mutations that bring neurodegeneration once introduced in C. elegans degenerins nor by low pH. However, it can associate both with MDEG1 and another recently cloned H⁺-activated channel DRASIC to form heteropolymers which display different kinetics, pH dependences, and ion selectivities. Of particular interest is the subunit combination specific for sensory neurons, MDEG2/DRASIC. In response to a drop in pH, it gives rise to a biphasic current with a sustained current which discriminates poorly between Na⁺ and K⁺, like the native H⁺-gated current recorded in dorsal root ganglion cells. This sustained current is thought to be required for the tonic sensation of pain caused by acids.

The protein MDEG (or BNaC1) was cloned from rat and human brain (1–3). It is a member of the degenerin/epithelial Na⁺ channel superfamily which includes sodium-permeable ion channels such as the epithelial Na⁺ channel (ENaC)³ (4–7), involved in sodium homeostasis as well as in taste perception (8), and the FMRFamide-activated Helix aspersa channel FaNaC (9), involved in neurotransmission. It also includes the degenerins of Caenorhabditis elegans, involved in mechanotransduction (10, 11). Rat MDEG did not display detectable currents after expression in Xenopus oocytes or HEK cells (1). In contrast, a large sodium-selective current was observed in cells that expressed mutated MDEG (1). The mutations correspond to substitution of a glycine, i.e. a small amino acid for a large amino acid, such as a valine or a phenylalanine, just before the second hydrophobic region. These mutations are equivalent to those which in the C. elegans degenerins cause cell swelling and neuronal death (10–12).

MDEG shares 67% sequence identity with the recently cloned ASIC channel (for acid sensing ion channel), another member of the ENaC/FaNaC/degenerin family (13). ASIC expression yields a transient amiloride-sensitive inward current in response to a rapid drop in extracellular pH, that is mostly carried by Na⁺ ions (13). It is found in both the central and peripheral nervous system (3, 13). More recently, a second H⁺-gated Na⁺ channel, called DRASIC, was found to be expressed specifically in dorsal root ganglion cells (14). Expression of DRASIC in either Xenopus oocytes or COS cells gives rise to a H⁺-inducible current with a dual time course comprising a rapidly inactivating current, followed by a slowly activating and sustained current (14). Both components are essentially Na⁺ currents.

Fast drops in extracellular pH have been shown to activate transient sodium currents in peripheral sensory neurons (15) and various central neurons (16, 17). Whereas the proton-gated current in brain neurons consists of a single rapidly inactivating component, a dual current is found in polymodal nociceptive dorsal root ganglion neurons (18). In these neurons, the transient H⁺-induced Na⁺ current is followed by a sustained component. This sustained current is most likely the base element in the perception of non-adaptive painful stimuli (19, 20). Although the biphasic kinetics closely resemble those of the DRASIC channel, differences remain concerning the selectivity of the sustained component of the current, the native one being a non-selective rather than a Na⁺-selective current.

In this study we will show that MDEG (now designated as MDEG1) expressed in COS cells corresponds to a proton-gated cation channel with properties different from ASIC or DRASIC. We will also describe the molecular cloning, localization, and functional expression of a splice variant of the MDEG1 subunit, MDEG2, which has major structural differences with MDEG1. MDEG2 is not active by itself, but it can associate with either

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† The abbreviations used are: ENaC, epithelial Na⁺ channel; ASIC, acid sensing ion channel; PFCR, polymerase chain reaction; PBS, phosphate-buffered saline; MES, 4-morpholinoothanesulfonic acid.

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MDEG1 or DRASIC, modifying their properties. In particular it will be demonstrated that this splice variant confers non-
selectivity to the late H+/induced current.

**EXPERIMENTAL PROCEDURES**

Cloning of MDEG2—A fragment of the expressed sequence tag (GenBank accession number W50528) was amplified by PCR and used to screen a mouse brain cDNA library (Stratagene). A clone of 3062 base pairs was sequenced on both strands. All nucleic acid positions in the text refer to identical or similar residues are printed white on black

| MDEG2 | 1 | MRS GGARLPAT AL GGC FRMARE OPAVAAARQPGGDS GDPA LQG |
|-------|---|-----------------------------------------------------|
| MDEG1 | 1 | MDL K -------- E SPS ------- EGS LQPST S10 FAN |
|       | 23 | PGVARR GPRSL SR TKL HLG RHC MCG A RAGCGS FORRAL W LAF CTSSL LGLL |
|       | 101 | LSWSN RRELY WLS FSPSH TRVHRE VS ROLP FPVTV CNYNPL RLF PRLS KCD |
|       | 151 | LYYA GHL GL LL PNTRAPL VSE LL RD EPRQWRKL ADFLR FLPP RH |
|       | 201 | EGI SAA FDLRGLHLEDMLLSCYRGC LCC PHNSS |
|       | 251/200 | EDGKPL LTTVKGCTNGLEI MLDI QQD EYLP WGETETTE FEAGVKVQIH |
|       | 301/250 | SQSEP PIQELFGEVAPG GTFVATQ ORLYLPPPWGCRRSESMGLDE |
|       | 351/300 | PVYSTI TACRI Dchter YI VENCNORMVHMGPADFT PEOKEHC EAPGL |
|       | 401/350 | LAEKJCNYC CLR PCI NTYNKEL SMVKI PSKTSAK ELYKFKNKSEKYIS |
|       | 451/400 | ENILVLDIFFEALNYEI EOEKA YVE VA ALLDGDI GQGOMFLIG ASLTL ILE |
|       | 501/450 | LF D YI YELI KEK LDDL LGK EEE EGS HDEN MST CDTM PNHSE TI SHTVNP |
|       | 551/500 | LQTA GTLEE I AC |

**Fig. 1. Sequence comparison between MDEG2 and MDEG1.** Alignment stops at position 237 of MDEG2 since after this residue the two sequences became identical. The bold lines represent the two putative transmembrane regions. External cysteines are marked with open circles. The amino acid that, after mutation, causes constitutive activation of MDEG1 and neurodegeneration in *C. elegans* when introduced into the degenerins is marked with an asterisk. Identical or similar residues are printed white on black or black on gray background, respectively. The sequences were aligned with the ClustalW program. The sequence of MDEG1 corresponds to GenBank accession number U53211.

MDEG2 5' probe, bases 154 to 612 for the MDEG1 5' probe, bases 217 to 1363 for the rat, and bases 1 to 1308 for the human MDEG1 probe overlapping MDEG2. The human blots were hybridized overnight at 65 °C in 5 × SSC, 10 × Denhardt’s solution, 0.1% SDS, 100 μg/ml fish sperm DNA, washed with 0.1 × SSC, 0.1% SDS at 70 °C and exposed to 20 °C until use. Two antisense synthetic oligonucleotides, complementary to the rat cDNA sequence of MDEG2 (B1 and B2) or MDEG1 (V and VI) were used to detect MDEG2 and MDEG1 transcripts. The sequences of the used oligonucleotides were the following: B1, 5′-ACATGTGCCGCAGCCCGTGCAATTTAGTGCGACT-3′ (nucleotides 196–232); B2, 5′-ACATGTGCCGCAGCCCGTGCAATTTAGTGCGACT-3′ (nucleotides 196–232); B2, 5′-ACATGTGCCGCAGCCCGTGCAATTTAGTGCGACT-3′ (nucleotides 196–232); B2, 5′-ATACAGCATGTCGACTGGTG-3′ (nucleotides 350–385); V, 5′-TCTCTGTG-3′ (nucleotides 53–90); V, 5′-ATACAGCATGTCGACTGGTG-3′ (nucleotides 350–385); V, 5′-TCTCTGTG-3′ (nucleotides 53–90); V, 5′-ATACAGCATGTCGACTGGTG-3′ (nucleotides 350–385); V, 5′-TCTCTGTG-3′ (nucleotides 53–90); V, 5′-ATACAGCATGTCGACTGGTG-3′ (nucleotides 350–385); V, 5′-TCTCTGTG-3′ (nucleotides 53–90); V, 5′-ATACAGCATGTCGACTGGTG-3′ (nucleotides 350–385); V, 5′-TCTCTGTG-3′ (nucleotides 53–90); V, 5′-ATACAGCATGTCGACTGGTG-3′ (nucleotides 350–385); V. 3′-end-labeled with [α-32P]dATP (3000 Ci/mmol, ICN Radiochemicals) by terminal deoxynucleotidyltransferase to an average specific activity of 1 × 107 cpm/μg. Sections were treated consecutively with 0.1% (w/v) proteinase K diluted in 0.1 M Tris, 50 mM EDTA (pH 8.0) for 15 min at 37 °C, 4% paraformaldehyde, PBS (pH 7.2) for 5 min. Slides were then rinsed 10 min in PBS, acetylated for 10 min in 0.25% acetic anhydride in 0.1 M triethanolamine, and dehydrated. Hybridization was carried out overnight at 37 °C in hybridization buffer (50% deionized formamide, 10% dextran sulfate, 500 μg/ml denatured salmon sperm DNA, 1% Denhardt, 5% Sacrose, 250 μg/ml yeast tRNA.
MDEG2 and MDEG1 mRNA. A, Northern blot analysis on human tissues. The probe used is a human probe similar to the rat probe 1 used in the part B, i.e. a probe overlapping a common region of MDEG1 and MDEG2 cDNAs. B and C, analysis of MDEG1 and MDEG2 mRNAs in rat brain. 10 μg of total rat brain RNA were loaded on each lane. Analysis were carried out using cDNA probes prepared, as shown in B, from 5′ parts of each clone and specific of each of the MDEG forms (probes 2 and 3) or with a probe revealing both MDEG1 and MDEG2 (probe 1) that has been used in A. The shorter transcript corresponds to MDEG1 and the longer one corresponds to MDEG2. M and MI, putative transmembrane regions; CRD, cysteine-rich domain. M, position mutated in MDEG1 and MDEG2.

20 mM dithiothreitol, 20 mM NaPO₄ in 2 × SSC, and the radiolabeled probe (at 0.2 ng/ml with specific activities of 8 × 10⁶ dpm/μg). After hybridization, slides were washed in 1 × SSC at room temperature for 30 min before dehydration, drying, and apposition to Hyperfilm βmax (Amersham) for 6 days. Slides were then dipped in Ilford K5 nuclear emulsion (diluted 1:1) and exposed 4 weeks. Sections were then stained with cresyl violet and coverslipped. The specificity of labeling was verified by in situ hybridization using cold displacement of the radioactive probe with a 500-fold excess of unlabeled oligonucleotide and by the use of two specific oligonucleotide probes targeted to non-conserved regions. MDEG2 expression in dorsal root ganglia was obtained from in situ hybridization with the double-stranded cDNA corresponding to bases 276–585 labeled with digoxigenin-dUTP and fluorescein-12-dUTP with PCR. Probe labeling, prehybridization, and immunological detection of digoxigenin transcripts with alkaline phosphatase-conjugated anti-digoxigenin antibodies was carried out following the protocols from Boehringer-Mannheim.

Expression and Electrophysiology in COS Cells—The MDEG1 and DRASIC coding sequences were amplified by PCR and subcloned in the PCI Vector (Promega) as described previously (13, 14). COS7 cells, at a density of 200,000 cells/35-mm diameter Petri dish, were co-transfected with the ASIC subunits and an expression vector containing the CD8 receptor cDNA (ratio 5:1) using the DEAE-dextran method. Cells were used for electrophysiological measurements 1–3 days after transfection. Successfully transfected cells were recognized by their ability to fix CD8 antibody-coated beads (23). Ion currents were recorded using either the whole cell or outside-out patch-clamp technique and stored on hard disk for later off-line analysis. The pipette solution contained (in mM): KCl 120, NaCl 30, MgCl₂ 2, EGTA 5, HEPES 10 (pH 7.2). For the “0 Na⁺” pipette solution, NaCl was replaced by KCl. The bath solution contained in mM: NaCl 140, KCl 5, MgCl₂ 2, CaCl₂ 2, HEPES 10 (pH 7.3). Changes in extracellular pH were induced by shifting one out of six outlets of a microperfusion system in front of the cell or patch. Test solutions having a pH of less than 6 were buffered with 10 mM MES rather than HEPES, but were identical in all other respects. Experiments were carried out at 22 ± 2°C (room temperature).

RESULTS AND DISCUSSION

One partial cDNA sequence from mouse embryo similar to that of MDEG1 cDNA was found in the data base of expressed sequence tags (GenBank accession number W50528). A frag-
A Splice Variant of the H⁺-gated Channel MDEG

The point of divergence between MDEG1 and MDEG2 cDNAs contains a potential splice junction. This strongly suggests alternative splicing that generates from a single gene, two transcripts coding for MDEG1 and the new protein MDEG2. The first 236-amino acid sequence of MDEG2 encoded by separate exon(s) shows significant similarities with MDEG1 (31% identity and 48% similarity). MDEG2 also possesses two putative transmembrane regions, one identical to MDEG1 (the C-terminal one) and one different from MDEG1 (the N-terminal one) (Fig. 1). Thus this protein has all the hallmarks of the ENaC/Churchward-Naan/ASIC/dengenerin family, i.e., two hydrophobic domains flanking a large region including a cysteine-rich domain that was shown to be extracellular for the epithelial Na⁺ channel (24–26) and for the dengenerin MEC-4 (27).

Northern blot analysis was performed on human tissues and on rat brain tissue with a probe overlapping the common region of MDEG1 and MDEG2 and/or with two probes specific of each splice variant (Fig. 2). The two mRNAs of 4.2 and 2.9 kilobases labeled by the common probe in human and rat brain (also shown by I–3) correspond to each splice variant, the shorter transcript corresponding to the MDEG1 form and the longer transcript corresponding to the MDEG2 form (Fig. 2, B and C). Of the tissues examined by Northern blot analysis, both splice variant forms are only expressed in brain (Fig. 2A). A more precise localization of MDEG1 and MDEG2 in the nervous system was assessed by in situ hybridization.

Analysis of the distribution of MDEG1 and MDEG2 mRNA was performed on x-ray film images and emulsion-coated sections of adult rat brains cut in sagittal and coronal planes (Fig. 3). The pair of 32P-labeled MDEG2 or MDEG1 oligonucleotides revealed identical labeling profiles within MDEG2 or MDEG1 experiments, respectively, and provided evidence of the signal specificity. MDEG2 transcripts were found in the majority of the neuronal perikarya but were not detected in glial cells. Higher expression levels appeared in the main olfactory bulb, neo- and allo-cortical regions, hippocampal formation, habenula, basolateral amygdaloid nuclei, and cerebellum (Fig. 3A, upper). In the olfactory system (Fig. 3B), cells expressing MDEG2 mRNA were localized in the glomerular cell layer, the internal granular layer, and the mitral and internal plexiform cell layers. Within the glomerular layer, transcripts were restricted to the periglomerular cells. In contrast, weak labeling was observed in the external plexiform layer and the olfactory nerve layer. In the neocortex, the large pyramidal neurons were strongly labeled in all cortical layers as well as in the small cells corresponding to oligo-, astro-, or micro-glia cells. In the hippocampal formation (Fig. 3C), MDEG2 mRNAs were highly expressed in dentate granule cells and hilar neurons, as well as in pyramidal cells of CA1-CA3 subfields. In addition, intense hybridization signals were observed in large interneurons located in stratum oriens and radiatum of all subfields. Within the thalamus, the medial and lateral habenula showed moderate levels of MDEG2 mRNAs. In the cerebellar cortex (Fig. 3D), Purkinje cells and granule cells displayed high levels of MDEG2 expression. The molecular layer was weakly labeled except in small neurons with a size and distribution similar to stellate and basket cells. No expression was seen in the large interneurons of all deep cerebellar nuclei. A diffuse expression was observed in the external granular layer, the internal granular layer, and the mitral and internal plexiform cell layers. Within the glomerular layer, transcripts were restricted to the periglomerular cells. In contrast, weak labeling was observed in the external plexiform layer and the olfactory nerve layer. In the neocortex, the large pyramidal neurons were strongly labeled in all cortical layers as well as in the small cells corresponding to oligo-, astro-, or micro-glia cells. In the hippocampal formation (Fig. 3C), MDEG2 mRNAs were highly expressed in dentate granule cells and hilar neurons, as well as in pyramidal cells of CA1-CA3 subfields. In addition, intense hybridization signals were observed in large interneurons located in stratum oriens and radiatum of all subfields. Within the thalamus, the medial and lateral habenula showed moderate levels of MDEG2 mRNAs. In the cerebellar cortex (Fig. 3D), Purkinje cells and granule cells displayed high levels of MDEG2 expression. The molecular layer was weakly labeled except in small neurons with a size and distribution similar to stellate and basket cells. No expression was seen in the large interneurons of all deep cerebellar nuclei. A diffuse expression was observed over most other regions including thalamic nuclei, substantia nigra, striatum, and globus pallidus of the basal ganglia, hypothalamus, midbrain, pons, and medulla. MDEG2 transcripts were expressed at low levels in choroid plexus. MDEG2 was localized in exactly the same brain areas as MDEG1 (Fig. 3A, upper and lower parts), raising the possibility that the two forms can be present in the same neurons and can interact with each other. High MDEG2 mRNA expression was
also observed in sensory neurons of the dorsal root ganglia (Fig. 3, E and F), while MDEG1 was not detected (not shown).

Mutations that cause gain of function in the \textit{C. elegans} degenerins and neurodegeneration are able to activate MDEG1 (1). These mutations are located just before the second hydrophobic region, \textit{i.e.} in a region where MDEG1 and MDEG2 sequences are the same (Figs. 1 and 2C). The two mutations G481F and G481K were introduced in MDEG2. While these mutations are able to activate MDEG1 (1), corresponding MDEG2 mutants were not active after expression in \textit{Xenopus} oocytes (not shown). Thus, differences in the N-terminal part of MDEG1 and MDEG2 which includes the first 236 amino acids and the first transmembrane domain have produced drastic differences between the properties of the two MDEG proteins.

The relatively high level of sequence identity between MDEG1 or MDEG2, and ASIC (67 and 55\% respectively) raised the possibility that MDEGs are proton-gated ion channels. Indeed, transfection of COS cells with MDEG1 yielded a proton-gated Na$^+$ current with properties different from both ASIC and DRASIC (Fig. 4, A and C). MDEG1 requires more acidic pH values for activation and starts to open at pH 4.5, a pH where ASIC and DRASIC are already maximally activated (13, 14). The inactivation kinetics of MDEG1 ($\tau_{\text{inact}} = 3.15 \pm 0.51 \text{ s}, n = 22$) are slower than for ASIC ($\tau_{\text{inact}} = 1.4 \pm 0.05 \text{ s}, n = 129; p < 0.001$). Transfection of COS cells with MDEG2 did not yield a proton-gated current, even at the very low pH of 3 (not shown). MDEG2 is inactive by itself.

Since MDEG2 and ASIC are present in the same tissues, \textit{i.e.} brain and dorsal root ganglion cells, coexpression of the two proteins in COS cells was then assayed. It gave H$^+$-gated currents that were not significantly different from those recorded with ASIC alone (not shown). Conversely, cotransfection of MDEG2 and MDEG1 lead to a new current that activated when the pH was shifted from 7.3 to 5 (Fig. 4B). The MDEG1/MDEG2 current induced by the acid shift to pH 5 displayed a slowly inactivating component that was not seen on expression of MDEG1 alone (Fig. 4A). Even after a period of 30 to 60 s following the change of pH, a small but persistent current could still be recorded from most cells expressing the MDEG1/MDEG2 mixture. Larger pH variations induced larger currents and at pH 3 the MDEG1/MDEG2 peak current had not attained its maximal value (Fig. 4C). This is in contrast with the MDEG1 current which saturated at a pH between 4.5 and 4 (Fig. 4C). These results suggest that MDEG2 interacts with MDEG1 to form heteromultimers with new properties. As the intensity of the MDEG1/MDEG2 peak current measured at pH 4.5 is only one-fifth of the MDEG1 peak current activated at the same pH (Fig. 4C), it is clear that most, if not all channels are expressed in the heteromultimeric form in cells transfected with both MDEG1 and MDEG2. No difference in steady-state inactivation of the peak current was noticed between MDEG1 and MDEG1/MDEG2 currents (Fig. 4D).

Unitary currents were recorded from outside-out patches excised from MDEG1-transfected cells and from MDEG1/ MDEG2-cotransfected cells. In both cases it was found that besides a main conductance state, subconductance states could be recorded (Fig. 4, E and F). The unitary currents reversed at 30 $\pm$ 2 mV for both MDEG1 ($n = 8$) and MDEG1/MDEG2 ($n = 11$) (Na$^+$ equilibrium potential = +40.1 mV) (Fig. 4F), indicating that the main MDEG1 and MDEG1/MDEG2 conductance states are mainly permeable to Na$^+$. The slope conductance between $-40$ and $+40$ mV is 13.4 $\pm$ 2 pS for MDEG1 and 13.7 $\pm$ 2 pS for MDEG1/MDEG2 (Fig. 4F).

Outside-out patches excised from COS cells were exposed to a drop in pH from the resting pH of 7.3 to pH 4. Na$^+$ was absent from the pipette solution and patches were clamped at +40 mV (Fig. 4, G and H). While the MDEG1 current remained inward during the pH pulse, the MDEG1/MDEG2 current gradually became outward. This result indicates that channel selectivities of the early transient component and of the delayed MDEG1/MDEG2 component are different. Both current components expressed by the MDEG1/MDEG2 association are completely inhibited by 0.5 mM of the epithelial Na$^+$ channel blocker, amiloride (Fig. 4H).

It has been shown previously that the DRASIC current, like the native proton-gated current in dorsal root sensory neurons, consists of two components: a rapidly inactivating current followed by a sustained current (14). However, unlike the native current, the two DRASIC components reverse at the same membrane potential of +32 $\pm$ 3 mV ($n = 5$) (Fig. 5, A and D). As MDEG2 is present in dorsal root ganglion cells, we tested the possibility that cotransfection of DRASIC and MDEG2 would yield a late non-selective current, while retaining an initial transient sodium-selective current. Fig. 5, B and E, show that coexpression of the two different subunits yielded a current which at first sight looked like a DRASIC-like current. The transient peak current produced by activation of DRASIC/ MDEG2 reversed at +40 mV with 30 mM Na$^+$ in the pipette ($n = 10$) (Fig. 5, B and E), while it reversed at a value well above +80 mV in the absence of intracellular Na$^+$ ($n = 5$) (Fig. 5, C and F). Hence the initial transient current is mostly carried by Na$^+$. The steady-state current, however, reversed
close to 0 mV in the absence or presence of Na$^+$, indicating that it is not a Na$^+$-selective current. It has been shown previously that the pH for half-maximum activation of the DRASIC current is at pH$_{H_2}$ = 6.5 (14). It was found that coexpression with MDEG2 did not change the pH dependence of the peak current (not shown). The fact that the sustained DRASIC/MDEG2 current reversed very close to 0 mV, independent of the Na$^+$ gradient, indicated that the sustained non-selective current due to the heteromultimeric assembly was probably not much contaminated by the presence of the homomultimeric DRASIC sustained current. It follows that the initial transient Na$^+$ peak current recorded from DRASIC/MDEG2 transfected cells must be a current resulting from the formation of a heteromultimeric channel. Hence, as in the case of the MDEG1/MDEG2 current, both the Na$^+$-specific and the non-selective cationic current flow through heteromultimeric channels. A situation where a single channel protein displays two different ion selectivities is not without precedents. It has been described previously for the purinergic P2X$_2$ receptor (28). What is the molecular basis for the capacity of the MDEG1/MDEG2 and the DRASIC/MDEG2 heteromultimers to generate 2 different types of currents? A first possibility is that a single channel species could gradually change its selectivity with time. Alternatively, the association of the different subunits could lead to different stoichiometric arrangements producing different types of channels, one “transient” and one “sustained.” Another possibility would be that the 2 different types of channel expression could be related to differences in phosphorylation states.

To see if the non-selective channel could be distinguished from its Na$^+$-selective counterpart by its unitary conductance, we systematically measured for the MDEG1/MDEG2 hetero-multimer all well resolved conductance states as a function of membrane potential. Fig. 4J shows the presence of many subconductance states that cannot be easily separated into two independent channel species, although it appears that in the range between 0 and +40 mV, both a Na$^+$-selective and a non-selective conductance might be present. These data do not permit a clear conclusion. However, since it was shown that the main 13.7 pS conductance is selective for Na$^+$, it seems likely that the non-selective current is associated with a channel of lower unitary conductance. In the DRASIC case, no systematic correlation could be found between the amplitudes of the peak and sustained current. In some cells the sustained current was almost absent (35 out of 85 cells, the sustained component was less than 10% of the peak current) while in others (8 out of 85 cells), the sustained component was at least 60% of the peak current. This observation might plead in favor of the hypothesis involving the formation of 2 different channels.

As for other ligand-gated ion channels (29), increased diversity in the H$^+$-gated channel family can be reached by alternative splicing and heteromultimeric assembly. MDEG2 is present in sensory neurons where it modulates the expression of DRASIC. Coexpression of the two proteins yields a H$^+$-gated current that now contains a non-selective sustained component. Thus, it seems very probable that these two units constitute at least part of the native proton-gated cationic channel of nociceptive neurons (18).

The expression of acid sensing ion channels is not restricted to sensory neurons. Like ASIC (14), both MDEG1 and MDEG2 are well expressed in the brain and MDEG1 is in fact only present in the brain. Because significant changes in extracellular pH have been shown to be associated with neuronal activity (30), the possibility that brain acid-sensitive ion-channels are involved in neurotransmission or neuromodulation does exist. However, the fairly acid threshold for activation of MDEG1 by pH does not seem to be readily compatible with a physiological role as an acid sensor. The MDEG1/MDEG2 heteromultimer also is only activable at very acidic pH. It is not known whether MDEG1 channels or MDEG1/MDEG2 heteromultimers in their native environment can associate with other subsidiary subunits or can be modified by factors such as phosphorylation to bring their pH sensitivity closer to pH ~7. Therefore, the possible functions of MDEG1 and/or MDEG1/MDEG2 channels in the brain remain unclear. Both MDEG1 and MDEG1/MDEG2 channels can probably be activated in pathophysiological situations such as ischemia and epileptic seizures which produce large acidifications of the extracellular medium (31). In such cases, the opening of these particular channels and especially the long lasting non-selective cation current produced by the MDEG1/MDEG2 heteromultimer would certainly have very deleterious effects contributing to neuronal cell death.

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