Molecular and Functional Characterization of Acid-sensing Ion Channel (ASIC) 1b*

Received for publication, May 4, 2001, and in revised form, June 25, 2001
Published, JBC Papers in Press, July 11, 2001, DOI 10.1074/jbc.M104030200

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Acid-sensing ion channels (ASICs) are activated by extracellular protons and are involved in neurotransmission in the central nervous system, in pain perception, as well as in mechanotransduction. Six different ASIC subunits have been cloned to date, which are encoded by four genes (ASIC1-ASIC4). Proton-gated currents have been described in isolated neurons from sensory ganglia as well as from central nervous system. However, it is largely unclear which of the cloned ASIC subunits underlie these native proton-gated currents. Recently, a splice variant, ASIC-β, has been described for ASIC1a. In this variant about one-third of the protein is exchanged at the N terminus. Here we show that ASIC-β has a longer N terminus than previously reported, extending the sequence divergence between ASIC1a and this new variant (ASIC1b). We investigated in detail kinetic and selectivity properties of ASIC1b in comparison to ASIC1a. Kinetics is similar for ASIC1b and ASIC1a. Ca2+ permeability of ASIC1a is low, whereas ASIC1b is impermeable to Ca2+. Currents through ASIC1a resemble currents, which have been described in sensory and central neurons, whereas the significance of ASIC1b remains to be established. Moreover, we show that a pre-transmembrane 1 domain controls the permeability to divalent cations in ASIC1, contributing to our understanding of the pore structure of these channels.

In recent years a family of H+-gated cation channels (acid-sensing ion channels; ASICs) belonging to the Mec/ENaC superfamily of ion channels has been cloned from neuronal tissues (1). Members of this supergene family form Na+-selective ion channels (PNa/PK: 8–40), which can be blocked by amiloride (IC50, 0.2–10 μM) (2, 3).

Members of the ASIC subfamily share only about 20–25% identity with the subunits of the epithelial sodium channel (ENaC) but show all the hallmarks of the supergene family including two hydrophobic domains, short N and C termini and a large loop containing conserved cysteines between the hydrophobic domains. Therefore, they share very likely also the topology with two transmembrane spanning domains and intracellular termini, which has been experimentally shown for ENaC subunits (4–6). There is good evidence for ENaC that amino acids preceding the second transmembrane domain (preM2 segment) and within the second transmembrane (M2) domain form the amiloride-binding site and the selectivity filter of this channel (7–10). Thus, it is likely that the pre-M2 and M2 domains of ASICs, which show considerable homology to ENaC subunits, also form part of the outer mouth of the pore.

One likely function of ASICs is the modulation of neuronal activity by extracellular pH (1). So far six different members of this subfamily have been cloned (ASIC1a, ASIC-β, ASIC2a, ASIC2b, ASIC3, and ASIC4), which are encoded by four genes. ASIC-β and ASIC2b are splice variants of ASIC1a and ASIC2a, respectively (11, 12). In both variants a similar part comprising about the first third of the protein is exchanged, whereas the C-terminal two-thirds are identical.

ASIC1a is expressed throughout the brain and in sensory neurons from the dorsal root ganglion (13), whereas ASIC-β is specifically expressed in sensory neurons (12). Both subunits form rapidly activating and completely desensitizing ion channels with activation through H+ in the physiological range (pH50 = 5.9–6.4). They form Na+-selective channels; ASIC1a but not ASIC-β is also permeable to Ca2+ (12, 13). As big parts of these proteins including pre-M2 and M2 domains are identical, other parts of the channel protein must be involved in determining ion selectivity. Difference in Ca2+ selectivity may be a major functional difference of these splice variants and may provide a means to control Ca2+ entry pathways in sensory neurons.

Here we show that ASIC-β has an unusually long N terminus; we call this new variant ASIC1b. ASIC1a and ASIC1b have similar activation and inactivation kinetics. Moreover we show that ASIC1a is characterized by a lower Ca2+ permeability than previously reported, and that a pre-M1 domain controls Ca2+ permeability of ASIC1. The properties of ASIC1a match well the properties of proton-gated channels, which had been described in isolated neurons, whereas the significance of ASIC1b remains to be established.

**EXPERIMENTAL PROCEDURES**

Cloning of cDNAs—We used conserved regions of the Mec/ENaC gene family to design PCR primers G95B-u (5'-GCCACSCWCAC1- MTSAYGG-3') and P153-l (5'-GAKRTTGCASABRGTSACRGCIGG-3'). Poly(A) RNA was isolated with QuickPrep Micro mRNA Purification Kit (Amersham Pharmacia Biotech, Uppsala, Sweden) from organ of Corti, stria vascularis, maculae, ampullae of semicircular canals, and the spiral ganglion of 3-day-old rats and reverse transcribed using SuperScript (Life Technologies, Karlsruhe, Germany). PCR was performed for 40 cycles as follows: 94 °C for 45 s, 54 °C for 45 s, 72 °C for 1 min.

*This work was supported by a grant of the Attempto research group program of the Universitätsklinikum Tübingen (FG 1-0-0) (to S. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EMBL Data Bank with accession number(s) AJ309926.

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†The abbreviations used are: ASIC, acid-sensing ion channel; ENaC, epithelial Na+ channel; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; MES, 2-(N-morpholino)ethanesulfonic acid; cRNA, complementary RNA.
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15 s. A 220-base pair PCR product showing homology to other members of the gene family was random primed with 32P-labeled and used to screen a vestibular system-specific cDNA library. Positive clones were isolated and the longest one (V10–11; 3.5 kilobases) was entirely sequenced on both strands (accession number AF009926).

Using the PCR amplified cDNA clone (CLONTECH, Palo Alto, CA), rapid amplification of cDNA ends (RACE) was performed with polya(adenosine) RNA from organ of Corti of 4–6-day-old rats or amputae of 4–7-day-old mice. Primers used were ASIC1b-5’ RACE 5’-CTGGCA-CAGGAAAGCCACGAGTGTCG-3’ and the nested PCR reaction ASIC1b-5’ RACE 5’-GCCACTGCCCATAAGGCCTGCC-3’. PCR products were subcloned using the TOP10-TA cloning kit (Invitrogen, Groningen, The Netherlands) and sequenced.

For expression studies, the entire coding sequence of ASIC1b was amplified from cDNA clone V10–11 by PCR using Expand High Fidelity (Roche Molecular Biochemicals, Mannheim, Germany) to yield clone ASIC1b. PCR primers were ASIC1b-5’-CCGCTGGACCCGGGCA-GATGCCGG-3’ and ASIC1b-3’-GGGTTAATCTAGAAGCAAGTTGCT-TCAGAG-3’. Using terminal restriction endonuclease recognition sequences (SacI and KpnI), the PCR product was ligated in the oocyte expression vector pSSSP containing the 5’-untranslated region from Xenopus β globin and a polya(T) tail. The resulting clone was verified by sequencing. Clone ASIC1b-M3 was obtained by ligating an EcoRV/ EcoO fragment of cDNA clone V10–11 in pSSSP. In this clone the first two methionines are missing.

ASIC1a was cloned from rat brain cDNA by PCR using Expand High Fidelity (Roche Molecular Biochemicals). Primers had been deduced from the published sequence for ASIC1a (13) and were ASIC1a-5’-CCGAGCTCTCCCTTGCAAGGATGAGTGGG-3’ and ASIC1a-3’-5’-GGGGATCTTCAAGTGTTAAGTCTGCTTCAAGG-3’. Using terminal restriction sites (SacI and KpnI), the PCR product was ligated in pSSSP and entirely sequenced on both strands.

Site-directed Mutagenesis—Chimeric molecules between ASIC1a and ASIC1b were constructed by recombinant PCR using Pwo DNA polymerase (Roche Molecular Biochemicals). Briefly, two fragments were amplified with primers containing an overlapping region, joined by recombinant PCR, digested by appropriate restriction endonucleases (chimeras C1-C6, BamHI/EcoRI; chimeras C7-C9, SacI/EcoNI; chimeras C10 and C11, SacI/VanII), and ligated into the corresponding cDNA in the vector pSSSP. All PCR-derived fragments were entirely sequenced.

Electrophysiology—Using mMessage mMachine (Ambion, Austin, TX), capped cRNA was synthesized by SP6 RNA polymerase from ASIC1a, ASIC1b, ASIC1b-M3, and chimeric cDNA, which had been linearized by EcoRI restriction enzymes. 1–10 ng of cRNA was injected into stage 5½–6 oocytes of Xenopus laevis and oocytes kept in OR-2 medium (concentrations in mM: 82.5 NaCl, 2.5 KCl, 1.0 NaHPO4, 5.0 HEPES, 1.0 MgCl2, 1.0 CaCl2, and 0.5 g/liter polyvinylpyrrolidone) for 1–5 days.

The bath solution for two electrode voltage clamp contained (in mM): 140 NaCl, 2.0 MgCl2, 1.8 CaCl2, 10 HEPES (standard measurements) or 50 BaCl2, 2.0 MgCl2, 10 HEPES (measurements determining permeability). pH was either 7.4 or 5.0 and adjusted using NaOH or Ba(OH)2. For pH 5.0, HEPES was replaced by MES buffer.

Rapid pH changes in the outside-out configuration were achieved by placing the patch in front of a piezo-driven double-barreled application pipette. Time constant for complete solution exchange was <2 ms. Leak currents in outside-out patches were estimated by running the fast voltage ramp also before and after activation by low pH. A mean value of both control measurements was then subtracted from the value measured during activation. Patch-pipettes contained (in mM): 140 KCl, 2.0 MgCl2, 5.0 EGTA, and 10 HEPES, pH 7.4; pH was adjusted using KOH. Bath solution for patch clamp experiments contained (in mM): 140 NaCl or LiCl, 2.0 MgCl2, and 10 HEPES, pH 7.4. Application solutions for bi-ionic conditions contained (in mM): 140 NaCl or LiCl or 70 CaCl2, 2.0 MgCl2, 1.8 CaCl2 (except the 70 CaCl2 solution), and 10 HEPES, pH 7.4 or 6.0. pH was adjusted using HCl, NaOH, LiOH, or Ca(OH)2.

Data Analysis—Whole cell currents were recorded with a TurboTec 01C amplifier (npi electronic, Tamm, Germany) and data analyzed using IgorPro software (WaveMetrics, Lake Oswego, OR). Currents from outside-out patches were recorded using an Axopatch 200A amplifier (Axon Instruments, Union City, CA). Data were sampled at 0.1 ms, filtered at 1 kHz and analyzed using Igor Pro. Holding potential was −100 mV.

Time constants for activation and desensitization were best fitted using a single exponential function. We analyzed measurements with Na+ as well as with Li+ in the application pipette.

The permeability ratio PNa/PK was calculated from the reversal potential using the Goldman-Hodgkin-Katz equation: Em = (RT/F)(ln(PNa/Na)+1/P[K−]), where T is absolute temperature, R the gas constant, and F the Faraday constant. We considered the effect of Mg2+ in the pipette and Ca2+ in the application solution negligible. PNa/PK was calculated from the change in reversal potential when Na+ was replaced by Li+. Primers used were ASIC1b-5’-GCCACTGCCCATAAGGCCTGCC-3’ and the nested PCR reaction ASIC1b-5’-GCCACTGCCCATAAGGCCTGCC-3’. PCR products were subcloned using the TOP10-TA cloning kit (Invitrogen, Groningen, The Netherlands) and sequenced.

The Faraday constant. We considered the effect of Mg2+ in the application solution using the following equation: DErev = Erev,Na − Erev,Cl = (RT/F)(ln(PNa/Na)+1/P[K−]−Ca2+) with PCl = PNa/(1 + eErev/Ca2+). Activity coefficient f of single ions of valence z was calculated for Na+ and Ca2+ using the equation: log f = −0.096(zF/P) + (P + 2)/2. The intrinsic strength I is defined as I = 0.5 S x C2.

In whole cell measurements with BaCl2 in the bath we determined the current amplitude in 10-mV steps between −30 and −100 mV. Mean of inward currents from uninjected oocytes was subtracted and reversal potentials were then calculated using a linear fit of the two current values between which current reversed its sign. Values are reported as mean ± S.D.

RESULTS

Cloning of ASIC1b—We isolated a cDNA clone for ASIC1b from a vestibular system-specific cDNA library. This clone contains a long open reading frame of 559 amino acids. The C-terminal part of the open reading frame is identical to the sequence of ASIC1a but the first 218 amino acids are different. Thus, this clone represents a splice variant of ASIC1a. ASIC1b is identical to ASIC-β described by Chen et al. (12) except that our clone is 46 amino acids longer at the N terminus. ASIC1b contains three methionines, which might initiate translation (Fig. 1). The third methionine corresponds to the first methionine of ASIC1a and is the initiator methionine described by Chen et al. (12) for ASIC-β. Sequence alignment of ASIC1b (accession number AJ309926) and ASIC-β (accession number AJ006519) reveals that there are two frameshifts, which can fully account for the different N termini, ruling out that both are independent splice variants. Therefore, we confirmed the 5’-sequence containing the two additional upstream methionines in ASIC1b by 5’-RACE. Several RACE products corresponded well to our cDNA clone extending it by only 26 base pairs (Fig. 1). They contained all three methionines in a single frame. Moreover, RACE analysis showed that the 5’ end of ASIC1b including the first and second but not third methionine is very well conserved in mice (Fig. 1). Human genomic sequences containing the gene for ASIC1 were identified by a BLAST search against the draft version of the human genome (accession number AC025154). The N terminus of ASIC1b has an uninterrupted orthologous mate on human chromosome 12. Again, the 5’ end of ASIC1b including the first methionine is well conserved. In contrast, the second methionine is replaced by a leucine and the third methionine is replaced by a lysine (Fig. 1).

Although we did not verify the human sequence for ASIC1b, it suggests that the long N terminus of ASIC1a is conserved in humans. In addition, the first methionine of ASIC1b is in a good surrounding for initiation of translation (“AAGATGC” in rat and mouse). Altogether, this methionine most likely represents the initiator methionine and ASIC1b represents the real splice variant of ASIC1a. We refer to the longer form using the first in-frame methionine as ASIC1b, and to the shorter form corresponding to ASIC-β as ASIC1b-M3. In addition to the different N termini, we found a threonine instead of a serine at position 12 of ASIC-β, a difference that had previously been noticed by others (16).

We investigated functional characteristics of ASIC1b by expression in Xenopus oocytes. ASIC1b expressing oocytes show full expression of proton-gated currents only after 4–5 days, whereas ASIC1b-M3 and ASIC1a expressing oocytes show full expression already 1–2 days after injection of cRNA, suggesting differences in assembly and/or targeting to the plasma membrane. Maximal expression level was in the order of 5–20 μA at
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pH 6.0 for ASIC1b, and up to 50 μA for ASIC1b-M3 and ASIC1a.

Analysis of Kinetics and Selectivity of ASIC1—Since protonated currents, which had been described in isolated neurons, show different kinetics, we first investigated activation kinetics of ASIC1a and ASIC1b. Using outside-out patches from oocytes, channels were rapidly activated by pH 6.0 (Fig. 2). Activation kinetics was well fitted by a single exponential function. The beginning of the rat cDNA clone containing ASIC1b is shown above the nucleotide sequence. Methionines are highlighted. Human genomic sequences coding for the human homologue of ASIC1b, which had been identified in the genome database (www.ncbi.nlm.nih.gov/genome/seq), are also indicated; only sequences following the first methionine are shown. Nucleotides of mouse and human ASIC1b, which are different from rat, are indicated by white letters on black background; amino acids, which are different from rat, are indicated below the respective nucleotide sequence. Rat and mouse ASIC1b are 96% identical between the first and third methionine of rat ASIC1b, and up to 50% similar between the first and third methionine of ASIC1a.

We then investigated ion selectivity under bi-ionic conditions— ASIC1b discrimination stronger between monovalent cations than ASIC1a. A typical example of a current trace is shown for ASIC1a activated with pH 6.0 in outside-out patches with a piezo-driven application pipette. A typical example of a current trace is shown for ASIC1a, and the total current in ASIC1a expressing oocytes. We used this differential permeability to Ba2+ in whole oocytes to determine the amino acids that are responsible for the permeability differences between ASIC1a and ASIC1b.

Structural Analysis of Divalent Cation Permeability—First, we constructed a series of chimeric channels, in which we replaced increasing parts of the N terminus of ASIC1a by the corresponding part of ASIC1b. In order to obtain high expression levels, we used the N terminus of ASIC1b-M3. Chimeras were all functional and showed current amplitude comparable to wild type ASIC1a. As is shown in Fig. 5, currents through chimeras, in which up to 19 amino acids were replaced, reversed around −65 mV, indicating that these are mainly carried by K+.

To qualitatively estimate permeability for divalent cations in whole oocytes, we used 50 mM BaCl2 in the extracellular solution and activated channels by pH 5.0. The endogenous Ca2+-activated Cl− channel of oocytes is less sensitive to Ba2+, thereby reducing artifacts. As is shown in Fig. 4a, proton-activated currents of ASIC1b expressing oocytes reversed under these conditions at −84.6 ± 3.6 mV (mean ± S.D.; n = 3), indicating that these are mainly carried by K+. Currents of ASIC1a expressing oocytes in contrast reversed at −65.0 ± 5.4 mV (n = 6), demonstrating a significant contribution of Ba2+ to the total current in ASIC1a expressing oocytes. We used this differential permeability to Ba2+ in whole oocytes to determine the amino acids that are responsible for the permeability differences between ASIC1a and ASIC1b.
Fig. 3. Ionic selectivity of ASIC1a and ASIC1b. Channels were activated with pH 6.0 in outside-out patches with the rapid application system. Once currents were fully activated, a fast (100 ms) voltage ramp was run from −100 to +100 mV and back to −100 mV and the reversal potential determined. The application pipette contained either 140 mM NaCl, 140 mM LiCl, or 70 mM CaCl2; the patch pipette contained 140 mM KCl. A typical example of an I/V curve is given for each condition. The two traces are from the two sides of the same voltage ramp. The indicated reversal potentials are the mean ± S.D. from five to nine independent experiments. Relative permeability ratios were calculated as described under “Experimental Procedures.” The presence of ASIC1b channels in outside-out patches when CaCl2 was used in the application pipette was verified by activation of the same channels also with NaCl in the application solution. Holding potential was −100 mV. The inset shows a current trace with voltage ramp.

Fig. 4. a, currents evoked in whole oocytes by stepping from pH 7.4 to 5.0 with 50 mM BaCl2 in the bath. Oocytes had been injected with cRNA for either ASIC1a or ASIC1b; uninjected oocytes served as a control. Holding potential is indicated. b, I/V relation. Data are from the same cells as in a. Mean of inward currents from uninjected oocytes (n = 3) had been subtracted from currents measured with ASIC1a (open circles) and ASIC1b (filled squares) expressing oocytes. Erev was calculated by doing a linear fit between the two values between which current reversed its sign. Mean values for Erev were −65.0 ± 5.4 mV for ASIC1a (mean ± S.D.; n = 23) and −84.6 ± 3.6 mV for ASIC1b (n = 3).

Next, we made a similar series of chimeras, this time replacing increasing parts of the N terminus of ASIC1b by the corresponding part of ASIC1a. Chimera 7, in which the first 62 amino acids of ASIC1b were replaced, showed a reversal potential of −84.7 ± 2.9 mV (n = 3) like ASIC1a wild type. Chimera 9, with 88 replaced amino acids showed a reversal potential of −64.3 ± 4.0 mV (n = 6) like ASIC1a wild type. Chimera 8, in which the first 70 amino acids of ASIC1b were replaced, and chimeras more distal to amino acid 99 showed very low level of expression and were therefore not further analyzed. Thus, these chimeras identify the same pre-M1 domain as the opposite chimeras, confirming that this domain controls permeability to divalent cations in ASIC1.

DISCUSSION

Functional Significance of ASIC1a and ASIC1b—We cloned a splice variant of ASIC1a from inner ear. A similar variant has previously been cloned from dorsal root ganglia by Chen et al. (12). We provide strong evidence that this variant has a longer N terminus than previously reported, extending the sequence divergence between ASIC1a and ASIC1b. The first 17 amino acids of this long N terminus of ASIC1b show significant similarity to the N terminus of ASIC4 (almost 50% identity) (17). They are followed by a stretch of repetitive amino acids. Such stretches often serve as flexible hinge regions in proteins. It might thus very well be that the N terminus of ASIC1b and ASIC4 constitutes a domain with so far unknown function. As ASICs have recently been implicated in the formation of mechanosensitive ion channels (18) and as such channels are generally believed to be associated with the cytoskeleton, one may speculate that this domain may mediate such interactions. Sequences of ASIC2a and ASIC2b diverge at the same position as ASIC1a and 1b, suggesting that evolution of the alternative splice form had occurred before the gene doubling that gave rise to ASIC1 and -2.

Various proton-gated currents, which differ with respect to their kinetics, ionic selectivity, threshold for activation by protons, and rate of recovery, have been described in isolated neurons (19–23). It is only beginning to emerge which of the cloned receptors underlie these currents. Of the cloned ASICs, ASIC2a is characterized by pH activation only in an unphysiologic range (24) and ASIC2b and ASIC4 are inactive by itself (11, 17). Therefore, if these subunits contribute to the formation of proton-gated channels, they will do so only through the...
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Proton-gated currents (threshold of current activation, pH 6.5) described in hypothalamic neurons show a kinetic very similar to ASIC1 (\(\tau_{\text{inact}}\), 6.48 ms; \(\tau_{\text{inact}}\), 1.28 s) (26). Since ASIC1b and ASIC3 are not significantly expressed in the central nervous system, this suggests that ASIC1a underlies this current. More recently it has been found that similar channels in mouse cortical neurons have no significant Ca\(^{2+}\) component and show a large cell-to-cell variation with respect to half-effective proton concentration (27). This shows that proton-gated currents are heterogeneous also in central neurons.

Ca\(^{2+}\) permeability of ASIC1a is low (\(P_{\text{Ca}}/P_{\text{Na}} = 18.5\)). Given that the extracellular Ca\(^{2+}\) concentration is much lower than the Na\(^{+}\) concentration, Ca\(^{2+}\) in-flow through ASIC1a will be very small under physiological conditions and will probably not lead to significant rise in intracellular Ca\(^{2+}\). It is therefore questionable whether the rather small difference in ion permeability between ASIC1a and ASIC1b will be of physiological significance. All time constants of activation or inactivation show only minor differences between the two splice variants. As synaptic transmission takes place in a few milliseconds, small differences in activation time constants as observed for ASIC1a and ASIC1b will not be of functional significance. Together, the significance of ASIC1b cannot be explained by its functional properties. It thus may constitute a component of a heteromeric channel.

Pore Structure of ASICs—Since the pore structure of ASICs and related channels is much less understood than that of other channel types, we addressed the structural basis for the observed difference in permeability for divergent cations. The pore structure of the related ENaC has been elucidated in some detail by detailed analysis of targeted mutations. This has identified amino acids in the pre-M2 and M2 segment as being important for block by amiloride (7) and formation of the selectivity filter (8–10). The corresponding amino acids are conserved in ASICs, suggesting that the overall pore geometry is similar. ENaC, though, is impermeable to Ca\(^{2+}\) and shows only a very low permeability to K\(^{+}\) (\(P_{\text{Na}}/P_{\text{K}} > 40\)). Moreover, pre-M2 and M2 domains are identical between ASIC1a and ASIC1b and cannot account for the differences in ion selectivity between these splice variants. For ASIC2a and ASIC2b, which are also identical in their C terminus including pre-M2 and M2, but which show different permeability ratios \(P_{\text{Na}}/P_{\text{K}}\), the difference in ion selectivity could be attributed to a short stretch of amino acids preceding the first transmembrane domain M1 (pre-M1 domain) (28). Our results demonstrate that the same pre-M1 domain controls Ca\(^{2+}\) permeability of ASIC1.

Strikingly, mutations of the pre-M1 segment in ENaC do not change ion selectivity but, rather, change the gating pattern of this channel leading to a gating mode characterized by a low \(P_{\text{Na}}\) (29, 30). Several models can be envisaged to explain the role of formation of heteromeric channels with other ASIC subunits. Recently, detailed investigation of the properties of ASIC3 showed that this receptor underlies the proton-gated current in cardiac afferents (16). This current is characterized by a fast kinetics (\(\tau_{\text{act}}\), <5 ms; \(\tau_{\text{inact}}\), 0.32 s) and very low Ca\(^{2+}\) permeability (\(P_{\text{Na}}/P_{\text{Ca}} > 100\)). Moreover, ASIC3 is more sensitive to protons than ASIC1a or ASIC1b (16). pH activation of ASIC1a and ASIC1b, though, resembles that of ASIC3.

Our study suggests that ASIC1a as well as ASIC1b have slower inactivation kinetics than ASIC3. In one of the initial studies by Krishtal and Pidoplichko (19), 25 neurons out of 67 isolated from the trigeminal ganglion showed a fast and completely desensitizing proton-gated current (\(\tau_{\text{inact}}\), 0.5–1 s), 2 neurons showed slower desensitizing currents (\(\tau_{\text{inact}}\), 3–4 s), and 5 neurons showed slowly and incompletely desensitizing currents. Later it was shown that the completely desensitizing current includes a Ca\(^{2+}\) component (\(P_{\text{Na}}/P_{\text{Ca}} \approx 3\)) (25). Although these data do not exactly match the properties of any recombinant ASIC, they suggest that channels formed by ASIC1a underlie some of the native currents in sensory neurons.

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of the pre-M1 domain. The outer vestibule of the ion pore is very likely formed by the pre-M2 domain (7) and the selectivity filter by amino acids in the M2 domain (8–10). The pre-M1 domain could form part of the inner mouth of the ion pore. In ENaC, the size of this inner mouth could be significantly larger compared with that of its selectivity filter, which would explain why mutations in this region do not change ionic selectivity in ENaC. In ASICs, however, this region may contribute to the selectivity filter. In a second model, based on the observation that mutations at the pre-M1 domain of ENaC have an effect primarily on the gating of this channel (29, 30), the pre-M1 domain could form part of the inner mouth of the ion pore. In ASICs, however, this region may contribute to the pore structure of ASICs. Conformational changes in the outer vestibule of the channel family will rely on characterizing the structure of the contribution of the pre-M1 domain to the pore of this ion channel. Although such a model is hypothetical at the moment, it seems clear that the pore structure of ASICs and probably ENaC is more complicated than previously anticipated.

Acknowledgments—We thank A. Rusch for help with the data analysis and H.-P. Zenner for generous support of this study.

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J. Biol. Chem. 2001, 276:33782-33787.
doi: 10.1074/jbc.M104030200 originally published online July 11, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M104030200

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