The acid-sensitive ion channel 1 (ASIC1a or BNaC2a) is the most abundant of all mammalian proton-gated ion channels and the one that has the broadest distribution in the nervous system. Hallmarks of ASIC1a are gating by external protons and rapid desensitization. In sensory neurons ASIC1 may constitute a nociceptor for pain induced by local acidification, whereas in central neurons it may modulate synaptic activity. To gain insight into the functional roles of ASIC1, we cloned and examined the properties of the evolutionarily distant species toadfish (Opsanus tau), —420-million year divergent from mammals. Analysis of the protein sequence from fish ASIC1 revealed 76% amino acid identity with the rat orthologue. The regions of highest conservation are the second transmembrane domain and the ectodomain, whereas the amino and carboxyl termini and first transmembrane domain are poorly conserved. At the functional level, fish ASIC1 is gated by external protons with a half-maximal activation at pH 5.6 and a half-maximal inactivation at pH 7.30. The fish differs from the rat channel on having a 25-fold faster rate of desensitization. Functional studies of chimeras made from rat and fish ASIC1 indicate that the extracellular domain specifically, a cluster of three residues, confers the faster desensitization rate to the fish ASIC1.

The acid-sensitive ion channels (ASICs) constitute a subfamily of the large epithelial sodium channel (ENaC)/DEG family of ion channels (17, 27). The ASIC1a protein (or BNaC2a) is the most abundant of all mammalian proton-gated ion channels and the one that is expressed in most neurons of the central and peripheral nervous systems (1, 2). The mammalian ASIC1, ASIC2, and ASIC3 are all activated by protons but the degree and rate of desensitization markedly differ in each type of channel. For instance, rat ASIC1 and ASIC3 exhibit rapid and complete desensitization at pH 5.0, whereas ASIC2 has a slow and incomplete desensitization, leaving a substantial component of persistent current in the continual presence of protons (29). Indeed, most of the functional differences in the currents generated by the ASICs can be attributed to differences in desensitization rate, suggesting that this property may be important in conferring specificity to the response of the various ASICs in different regions of the nervous system. Numerous functions have been proposed for ASIC1 including a role in sensory transduction, specifically in nociception (pain induced by ischemia and inflammation) (7, 9, 15, 20, 21, 24), and as modulators of synaptic transmission and long term plasticity (2, 28). For many of these functions, in particular nociception, proton sensitivity plays a fundamental role in the physiology of these channels.

In order to gain more insight in the structure-function of these channels, we cloned and examined the functional properties of ASIC1 from an evolutionarily distant species, the fish Opsanus tau. Comparison of the properties of the fish and mammalian ASIC1 revealed significant differences, primarily Ca²⁺ dependence for activation and faster desensitization rate of the fish ASIC1. To identify the structural determinants of the desensitization process, we constructed a series of chimeras with the cDNAs of fish and rat and expressed them in Xenopus laevis oocytes for functional analysis with the two-electrode voltage clamp and patch clamp techniques.

MATERIALS AND METHODS

Cloning of Fish ASIC1 cDNA—Poly(A)⁺ was extracted from brain and spinal cord of toadfish using oligo(dT)-cellulose and proteinase K (Roche Applied Science) as described (8). First strand cDNA synthesis was performed using oligo(dT) primers and SuperScript reverse transcriptase (Roche Applied Science). We designed degenerated primers from highly conserved sequences among all the mammalian ASIC proteins. We selected the protein sequence NCNRCMHMPG to make the sense primer: AA(C/T)TG(C/T)AG(A/G)ATGGTICA(C/T)ATGCCIGG. The antisense primer was designed from the protein sequence GDIG-GGQM: CCCAT(C/T)TGCCICICATAG/CTGCIC, where I indicates inosine. The position of the primers is indicated in Fig. 1. PCR was performed on first strand cDNA with Tq polymerase (Roche Applied Science) with the following parameters: denaturing for 20 s at 94 °C, annealing for 30 s at 55 °C, and extension for 30 s at 72 °C, repeated for 30 cycles. The PCR product was sequenced and used to design specific primers for 5'- and 3'-rapid amplification of cDNA ends performed with 5'- and 3'-rapid amplification of cDNA end system kits (Roche Applied Science) according to the protocols provided by the supplier. Once the 5'- and 3'-untranslated sequences were obtained, we designed a new set of oligonucleotides to amplify the whole DNA coding sequence using high fidelity Tq plus Tgo polymerases (Roche Applied Science). To the antisense 3' primer was added the sequence of the HA epitope, such that translation of the final cDNA produced a protein with the HA epitope in the carboxyl terminus. The PCR product was subcloned in PCR.2 vector (Invitrogen). Several clones were sequenced with an automatic DNA sequencer at the Keck Facility at Yale University.

Construction of Rat and Fish ASIC Chimeras and Site-directed Mutagenesis—Fish and rat ASIC1 chimeras were made by PCR using hybrid primers and a combination of Tq and Tgo DNA polymerases (Roche Applied Science) as described (14). Constructs were subcloned in pCDNA-3.1 vector (Invitrogen). Site-directed mutageneses of cDNAs were performed with the QuickExchange kit according to the manufacturer's instructions (Stratagene). All constructs underwent DNA sequencing at the Keck Facility of Yale University prior to further use.

Synthesis of cRNA and Injection of X. laevis Oocytes—cRNAs were

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FIG. 1. Sequence alignment of fish and rat ASIC1 proteins. The protein sequences were aligned with the ClustalW program. Black shading, identical amino acids; gray shading, conserved amino acids. The first and second transmembrane domains are indicated by bars, M1 and M2. The dashed lines correspond to the position of the sense and reverse degenerated primers used for the initial amplification.

synthesized with T7 RNA polymerase from linearized plasmids containing the entire coding sequence of the genes using mMESSAGEniMACHINE (Ambion). Stage V and VI oocytes were injected with 2 ng of cRNA from rASIC1, fASIC1 or each of the chimeras. Oocytes were incubated in frog Ringer: 96 mM NaCl, 2 mM KCl, 1 mM MgCl2, 5 mM HEPES, adjusted to pH 7.4 for 2–3 days at 19 °C before making recordings. Prior to patching, oocytes were placed in an hypotonic solution: 220 mM N-methyl-D-glucamine, 220 mM aspartic acid, 2 mM MgCl2, 10 mM EGTA, 10 mM HEPES, adjusted to pH 7.4 with KOH to remove the vitelline membrane with fine forceps.

Single-channel Recordings—Unitary currents were recorded using the outside-out configuration of the patch clamp technique. Channels were activated by rapidly moving squared glass tubes (inner diameter 0.7 mm) delivering solutions of desired pH values in front of the tip of the patch pipette. The delivery device achieves complete solution changes within 20 ms (SF-77B; Perfusion Fast-Step, Warner Instruments Corp., Hamden, CT). Pipettes were pulled from borosilicate glass (LG16; Dagan Corp.) using a micropipette puller (PP-83, Narishige; Scientific Instrument Laboratory) and fire-polished to a final tip diameter of 1 μm. When pipettes were filled with solutions, the resistance was 5–10 megohms. Single channel currents were recorded with an Axopatch-200B amplifier (Axon Instruments) using a DigiData 1200 series interface and pClamp 8.3 software, both from Axon Instruments. Data were recorded at 10 kHz, filtered at 5 kHz, and stored on a computer hard disc for analysis. For display, data were filtered with a digital Gaussian filter to 0.5 kHz. Pipette solution (intracellular) was as follows: 150 mM NaCl, 1 mM MgCl2, 5 mM HEPES, adjusted to pH 7.4 with KOH — 1 represents the component of the current that inactivates with the rate constant λ (s−1), and M0 is a constant that accounts for the offset of the current.

RESULTS

Cloning of ASIC1 cDNAs from Toadfish—To clone the fish ASIC1, we identified highly conserved sequences among all of the mammalian ASIC proteins cloned to date. We designed two degenerated primers corresponding to the amino acid sequences NCNCRMVHMPG (sense) and GDIGGQMG (reverse) that are highly conserved. These primers were used to amplify a 360-base pair fragment from single strand cDNA synthesized from poly(A)− extracted from adult toadfish brain and spinal cord. The DNA sequence of the PCR product indicated that it corresponded to the fish ASIC1 gene. The complete coding sequence of the cDNA was then obtained using 5′ and 3′-rapid amplification of cDNA ends as described under “Materials and Methods.”

Fig. 1 shows an alignment of rat (26) and fish ASIC proteins. The two proteins exhibit 76% amino acid identity. The domains of highest homology are M2 (90.6%) and the ectodomain (82.4%), whereas the cytoplasmic amino terminus (53.3%), carboxyl terminus (58.2%), and M1 (59.4%) are more divergent.
Functional Expression of Fish ASIC1 in Xenopus Oocytes—
Fish ASIC1 cRNA was injected in Xenopus oocytes, and proton-activated currents were measured 2 days after injection with the two-microelectrode voltage clamp. Fig. 2A shows representative whole-cell inward currents elicited by a change in pH, from 7.4 to 5.0. The current has a rapid onset of activation and desensitizes rapidly in the presence of protons. After electrophysiological measurements, the same oocytes were homogenized and treated for Western blotting. The FASIC1 protein was revealed with anti-HA monoclonal antibody. A protein of 65 kDa was identified in injected oocytes but not in controls (Fig. 2B).

Unitary Currents of Fish ASIC—We then examined the properties of unitary currents of fish ASIC1 expressed in X. oocytes with the patch-clamp technique in the outside-out configuration. Channels were activated by a rapid change of external pH, from 7.4 to 5.0. Fig. 3A shows representative examples of fASIC1 recorded in symmetrical 150 mM NaCl. For comparison, Fig. 3B shows traces from rASIC1 obtained under identical experimental conditions. The spikes in the initial part of the records represent noise introduced by the perfusion system when the solutions are changed. After a brief delay, fASIC1 channels open almost synchronously, followed by rapid and progressive closure in the continual presence of external protons. The records reveal uniform amplitude of the fish unitary currents in contrast to the rASIC1, which exhibits subconductance states in addition to the fully open channel. Subconductances are prominent at the end of the bursts of activity in the middle and lower traces from Fig. 3B (21). There are also significant differences in channel kinetics. Whereas the open state of fASIC1 is rarely interrupted by closures, rASIC1 exhibits complex kinetics with multiple brief openings and closures throughout the burst of activity that confer a flickering appearance to the traces.

Another significant difference between these channels is the shorter duration of the bursts of activity of the fish than the rat channels. Notice the 20-fold difference in the time scales in Fig. 4, A and B. This finding indicates that the desensitization rate of fish is faster than rASIC1.

Desensitization rate constants were determined from the sum of 12 independent patch clamp records from fASIC1 or rASIC1. The continuous lines in Fig. 4 represent the fit of the data with a single exponential. The calculated desensitization rate constants were 56 and 2.0 s⁻¹ for the fish and rat, respectively.

Properties of FASIC1: Apparent Affinity for Proton Activation and Desensitization, Single Channel Conductance, Ion Selectivity, Amiloride Block, and Temperature Sensitivity—To determine the affinity of fASIC1 for external protons, we calculated the apparent half-maximal activation from peak whole-cell currents elicited by progressively lowering the pH using TEVC. The protocol consisted of 30-s perfusion with preconditioning pH 7.4 before the external solution was changed to each of the test solutions. Peak currents were normalized to the maximal value obtained with a pH of 4.0. The line in Fig. 5A represents the fit of the data to the Michaelis-Menten equation with calculated half-maximal proton activation pH₅₀ of 5.6 ± 0.4.

The effect of preconditioning pH₄ was assessed by measuring peak currents induced by changes in solutions from a series of preconditioning pH₄ values to a solution of pH₄ of 5.5 (Fig. 5B). The dependence of desensitization on proton concentration, pH₅₀, was calculated to be 7.36. The steep slope of the curve indicates that desensitization takes place by a cooperative process.

The conductance of fASIC1 unitary currents were determined from I-V curves recorded in the presence of 150 mM Na⁺ or 150 mM Li⁺ and with 1 mM Ca²⁺ only in the external solutions. Fig. 5C shows linear I-V relations as predicted by the voltage-independent behavior of the ASIC family of ion channels. Currents were of larger magnitude in the presence of Na⁺ than in Li⁺. The cord conductance, calculated in the voltage
range of −20 to −120 mV, was 29 and 20 pico siemens for Na⁺ in Li⁺, respectively. In the presence of K⁺, the unitary currents were much smaller than in Na⁺, and when Ca²⁺ was the only external cation, the amplitudes were too small to be discernible as individual transitions (not shown).

A characteristic of the ENaC/DEG channels is the block by amiloride or its analogues. The half-maximal concentration for inhibition of fASIC1 was calculated by measuring peak currents induced by a change in pH from 7.4 to 5.5 in the presence of increasing concentrations of external amiloride. The calculated $K_{D}$ was 9.6 μM, and the Hill coefficient was 0.9 (Fig. 5D).

The apparent affinity for external Na⁺ was estimated by measuring peak currents induced by pH 5.0 in the presence of increasing concentrations of external Na⁺ from 30 to 300 mM. The osmolarity of the solutions was kept constant, 600 mMols, by the addition of mannitol. The data were fitted to the Michaelis-Menten equation with a $K_{D}$ value of 118 mM (Fig. 5D).

A previous report indicated that low temperature slows the rate of desensitization of the mammalian ASIC1 (2). It was suggested that this property could modulate the activity of the ASICs in peripheral terminals where the temperature is a few degrees lower than in the central nervous system. In contrast to mammals, the body temperature of fishes is lower, and it fluctuates over a much larger range than in mammals, making possible modulation of the activity of fASIC1 not only in the periphery but also in the central nervous system. We therefore investigated whether the activity of fASIC1 is sensitive to changes in temperature. Fig. 5F shows traces of whole-cell currents activated by pH 5.0 at 22 or 6 °C, which is the normal range of temperatures experienced by most marine fishes. Peak currents and the rate of desensitization did not differ significantly at 22 or 6 °C.

**External Ca²⁺ Is Required for Proton Activation of fASIC1**

External Ca²⁺ has been implicated in the modulation of the rat ASIC1 (10, 19, 21) and of ASIC3 (12). We examined the effect of external Ca²⁺ on the activity of fASIC1 by first measuring whole cell currents with 1 mM external Ca²⁺ or nominal free Ca²⁺ (no EDTA added). Fig. 6A shows records of fASIC1 whole-cell currents activated by a change in pH from 7.4 to 5.0 with 1 mM or without Ca²⁺. The concentration of external Ca²⁺ and the pHs are indicated above the current traces. In the initial part of the experiment, the oocyte was perfused with 1 mM Ca²⁺ in the preconditioning (pH 7.4) and activating (pH 5.0) solutions. A robust response was elicited, which was considered to be the maximal response. The second trial shows a similar experiment but without Ca²⁺ in the solutions. Here, pH 5.0 failed to activate fASIC1. When the preconditioning solution had 1 mM Ca²⁺ and the activating solution had 0 mM Ca²⁺, a current of −80% of the maximal response was elicited. Finally, if the preconditioning solution did not contain Ca²⁺ and the
activating solution had 1 mM Ca\textsuperscript{2+}, the current was ~20% of the maximal response. The results were reproduced independently of the sequence on which the four different pairs of solutions were applied. These experiments indicate that Ca\textsuperscript{2+} must be bound to the channel prior to the arrival of protons in order to achieve maximal activation.

The apparent affinity for external Ca\textsuperscript{2+} was estimated by measuring whole-cell currents elicited by pH\textsubscript{o} 5.0 solutions with increasing concentrations of Ca\textsuperscript{2+}. For each tested Ca\textsuperscript{2+} concentration, the preconditioning and activating solutions contained the same amount of Ca\textsuperscript{2+}. Fig. 6B shows a plot of normalized peak currents, with the line representing the fit of the data with half-maximal activation constant of 3 mM and Hill coefficient of 1.9.

We also observed that when the concentration of Ca\textsuperscript{2+} was increased above 1 mM, the activity of fASIC1 decreased progressively, but it was not completely abolished even when external Ca\textsuperscript{2+} concentration was raised to 60 mM.

To investigate the nature of Ca\textsuperscript{2+}-induced inhibition, we examined the effects of increasing Ca\textsuperscript{2+} concentrations on the unitary currents. Outside-out patches were perfused with pH\textsubscript{o} 5.0 solutions containing 150 mM Na\textsuperscript{+} and increasing concentrations of Ca\textsuperscript{2+}. Representative examples in Fig. 6C show a progressive decrease in the amplitude of the unitary currents. A plot of the magnitude of normalized unitary currents to 0 mM external Ca\textsuperscript{2+} is shown in Fig. 6D, where the line represents the fit of the data to the Michaelis-Menten equation with values for K\textsubscript{D} of 16 mM and n of 1.3.

**Determinants of the Kinetics of Desensitization of ASIC1**—One of the main functional differences between fish and rat ASIC1 is the rate of desensitization. Since these two channels exhibit a high degree of amino acid conservation, we thought that it would be possible to identify the sequences conferring this property by swapping domains between the fish and rat ASIC1. First we examined the contribution of the five defined structural domains of ASIC1: amino terminus and carboxyl terminus, M1 and M2, and the ectodomain. Fig. 7 shows a schematic representation of the first set of chimeras, where shown in white are sequences from fish ASIC1, in black are sequences from rat ASIC1, and in gray are sequences from rASIC2. The numbers under the chimeras indicate the amino acids in the protein sequence corresponding to the rat or fish ASIC. The cRNA of each chimera was injected in oocytes, and whole-cell and unitary currents were examined. The seven chimeras were functional when expressed in oocytes. The rate of desensitization (\(\lambda_{des}\) s\textsuperscript{-1}) was measured from currents elicited by pH\textsubscript{o} 5.0 and 1 mM Ca\textsuperscript{2+}. CH1 and CH2 were designed to test the contribution of the transmembrane domains. These chimeras desensitized at the rates corresponding to fish and rat ASIC1, indicating that the transmembrane domains do not.
influence the $\lambda_D$. CH3 and CH4 were designed to test the contribution of the amino and carboxyl termini. These chimeras exhibited desensitization rates of the fish and rat ASIC1, respectively, indicating that the intracellular domains of the channel do not contribute significantly to desensitization. Finally, swapping only the extracellular domains, CH5 and CH6, reproduced the desensitization rates of fish and rat ASIC1, respectively. We confirmed the importance of the extracellular domain by making CH7, which contains M1, M2, and amino and carboxyl termini from the rapid desensitizing fish ASIC1 ($\lambda_D$ of 56 s$^{-1}$) and the ectodomain from rASIC2, which desensitizes very slowly and incompletely ($\lambda_D < 0.02$ s$^{-1}$) (20). CH7 exhibited a slow and incomplete desensitization rate, indistinguishable from wild-type rASIC2, confirming the crucial role of the ectodomain in determining $\lambda_D$. Fig. 7B shows representative examples of patch recordings of the three chimeras, where the whole ectodomain has been exchanged.

To define further the regions in the extracellular domain important for desensitization, we made additional chimeras, swapping shorter segments of the ectodomain, CH8 to CH15 (Fig. 8).

CH8, which contained the carboxyl terminal half of the ectodomain from rats, exhibited a fast $\lambda_D$ of 24 s$^{-1}$, whereas CH9, which contained the amino terminal half of the ectodomain from rats, exhibited a much slower $\lambda_D$ of 5.0 s$^{-1}$. This result indicates that the first half of the ectodomain of fish confers the faster $\lambda_D$. CH10 ($\lambda_D$ of 20 s$^{-1}$) and CH11 ($\lambda_D$ of 4.4 s$^{-1}$) maintained the same properties as the previous two chimeras; therefore, the segment comprised between amino acids 76 and 130 is important to determine the $\lambda_D$. Additional chimeras, CH12 to CH15, confirmed this observation. Fig. 8B shows representative examples of the most informative chimeras.

A sequence comparison of the segment comprised by amino acids 76–130 from rat and fish is shown in Fig. 9A. Boxes surround nonconserved residues. We exchanged the nonconserved residues in the rat ASIC1 to generate rASIC-PLM and rASIC-HLVE mutants. The rASIC1-PLM channel exhibited a desensitization rate of 23.1 s$^{-1}$, much faster than the wild type rASIC1, whereas the mutant rASIC-HLVE had a desensitization rate no different from wild type. Conversely, substitution of the corresponding residues from rat into fASIC1, fASIC1-SQL, resulted in a significant reduction of $\lambda_D$ (4.6 s$^{-1}$). Thus, substitution of three residues in the extracellular domain of the rat channel for the corresponding ones in fish is sufficient to induce a fast desensitization. However, single amino acid mutations produce only small changes in $\lambda_D$, indicating that the desensitization rate depends on the synergistic effect of the three residues.

DISCUSSION

Structural and Functional Comparison of Fish and Rat ASIC1—Comparison of the sequences of fish and rat ASIC1 reveals 76% overall identity at the amino acid level. Most of the differences are confined to M1 and the cytoplasmic domains, whereas M2 and the ectodomain are highly conserved (27).

We found that the pore properties of the channels from the two species are identical: ion selectivity (Na$^+$ > Li$^+$ > K$^+$ [tmt])
Ca\(^{2+}\)) single channel conductance (29 and 30 picosiemens for fish and rat), and amiloride block (K\(_{i}\) of 9.4 and 10 \(\mu M\) for fish and rat) (29). These functional results together with the highly conserved sequence of M2 support the view that M2 forms the ion pathway (17, 18). Although the region preceding M1 has also been implicated in ion permeation (11), we found little conservation of the poorly conserved amino termini with the pore properties.

The other region that exhibits a high degree of identity is the extracellular domain (82.4%). Sequence conservation of extracellular domains is not a characteristic of all members of the ENaC/DEG family. For instance, the subunits of ENaC from rat and frog share only ~42% identity, although the functional properties of ENaC from these two species are indistinguishable (22). Thus, the high degree of conservation of the ectodomain suggests that it is particularly important in the ASIC family. Indeed, this domain has been implicated in the gating mechanism by binding external protons and Ca\(^{2+}\) but in contrast to the rASIC1, the fish channel requires more external Ca\(^{2+}\) for activation (\(K_{D}\) of 3 \(\mu M\)).

Another important difference between the fish and mammalian channels is the desensitization rate. Desensitization is a process that occurs in most ligand-gated channels. The rate and extent of desensitization are important properties that shape the amplitude, duration, and frequency of the channel response. A 25-fold faster desensitization rate implies that for the same drop in pH\(_{50}\), the amount of charge entering into fish neurons is significantly smaller than in mammalian neurons. The consequences of this effect on the physiology of ASIC1 in the nervous system are not apparent with our current state of understanding of these channels. However, in the last few years, it has been shown that a host of different substances and conditions modulate the rate and extent of desensitization of the mammalian ASICs: external protons (29), Ca\(^{2+}\) (13, 16, 29), Zn\(^{2+}\) (5), neuropeptides (4, 12, 25), combinations of various subunits (6), mutations similar to the ones in the degenerins (26), and temperature (3). Despite these observations, nothing is known about the molecular mechanisms underlying the desensitization of ASIC1 or the structural domains that participate in the process.

To gain insight into these questions, we took advantage of the differences in desensitization rates between fish and mammalian ASIC1 to identify structural determinants that influence \(\lambda_{D}\). Our results derived from functional analysis of many chimeras indicate that the extracellular domain determines the rate of desensitization of ASIC1. Further dissection of this domain led us to identify a short sequence constituted by three residues (Ser\(^{35}\)-Gln\(^{35}\)-Leu\(^{35}\) in rat and Pro\(^{35}\)-Leu\(^{35}\)-Met\(^{35}\) in fish) that is responsible for most of the difference in \(\lambda_{D}\) observed in rat and fish ASIC1. The full effect of the three residues was not reproduced by single mutations in any of the three positions, but together they had a synergistic effect. The effect was specific, because swapping of other amino acids in the same region, the quadruple mutations, QMAD\(^{131}\) for HLVE\(^{131}\), or the single mutation, Glu\(^{35}\) → Ser did not alter desensitization (not shown). We did not substitute all of the different amino acids in the segment comprised by residues 76–130, because the effect of the triple mutant R-PLM increased the \(\lambda_{D}\) from 2.2 to 20 s\(^{-1}\) to the same degree as the swapping of the whole segment (CH10 and CH13 \(\lambda_{D}\) of 20 and 17.1 s\(^{-1}\), respectively). Most importantly, other properties of the channels were not affected, such as the pH\(_{50}\) of activation or rate of recovery from desensitization (data not shown).

The mechanism underlying the changes in \(\lambda_{D}\) cannot be determined by our data; however, we can speculate that these amino acids may mediate a conformational change that determines the transition from the open to the desensitized state (hinge hypothesis). Alternatively, in the open state, these residues may come in contact with another region of the protein such that the interaction stabilizes the open state and slows the desensitization rate. On the contrary, if the interaction destabilizes the open state, the desensitization rate becomes faster (interface hypothesis). Although the triad of amino acids we have identified is important, there are additional residues in the ectodomain that influence the rate of desensitization, because substitution of these residues did not completely restore the \(\lambda_{D}\) to the value of the wild-type channels.

An additional important conclusion from these experiments is that activation and desensitization are independent processes, because variations in \(\lambda_{D}\) did not change the apparent pH\(_{50}\) of activation in wild type fish and rat ASIC1 or in the chimeras.

In summary, functional analysis of two evolutionarily distant ASIC1 proteins has provided the first evidence that the
ectodomain, in particular a short sequence of three residues, determines the desensitization process of ASIC1.

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The Extracellular Domain Determines the Kinetics of Desensitization in Acid-sensitive Ion Channel 1
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