PSD-95 and Lin-7b Interact with Acid-sensing Ion Channel-3 and Have Opposite Effects on $H^+$-gated Current*

The acid-sensing ion channel-3 (ASIC3) is a degenerin/epithelial sodium channel expressed in the peripheral nervous system. Previous studies indicate that it participates in the response to mechanical and painful stimuli, perhaps contributing to mechanoreceptor and/or $H^+$-gated nociceptor function. ASIC3 subunits contain intracellular N and C termini that may control channel localization and function. We found that a PDZ-binding motif at the ASIC3 C terminus interacts with four different proteins that contain PDZ domains: PSD-95, Lin-7b, MAGI-1b, and PIST. ASIC3 and these interacting proteins were expressed in dorsal root ganglia and spinal cord, and PSD-95 co-precipitated ASIC3 from spinal cord. When expressed in heterologous cells, PSD-95 reduced the amplitude of ASIC3 acid-evoked currents, whereas Lin-7b increased current amplitude. PSD-95 and Lin-7b altered current density by decreasing or increasing, respectively, the amount of ASIC3 on the cell surface. The finding that multiple PDZ-containing proteins bind ASIC3 and can influence its presence in the plasma membrane suggests that they may play an important role in the contribution of ASIC3 to nociception and mechanosensation.

ASIC3 is a non-voltage-gated Na+ channel activated by acidic extracellular solutions (1, 2). It is expressed in the peripheral nervous system, including the dorsal root ganglia and trigeminal ganglia. Immunocytochemical studies have localized it to several different specialized sensory nerve endings of skin, suggesting it might participate in mechanosensation and nociception (3). The importance of ASIC3 for sensory function was revealed by studies of mice bearing targeted disruptions of the ASIC3 gene (3, 4). In single fiber recordings from cutaneous nerves, loss of ASIC3 increased the sensitivity of mechanoreceptors detecting light touch but reduced the sensitivity of a mechanoreceptor responding to noxious pinch. In behavioral studies, ASIC3 modulated the response to noxious acid, heat, and mechanical stimuli. Moreover chronic mechanical hyperalgesia produced by intramuscular acid injections was prevented in ASIC3 null animals (5). Functional studies suggest that ASIC3 may also mediate the pain associated with myocardial ischemia (6, 7). Thus, in different cellular contexts, ASIC3 may participate in the responses to both mechanical and acidotic stimuli to mediate normal touch and pain sensation.

ASIC3, a member of the DEG/ENaC family, forms heteromultimers in neurons with other DEG/ENaC subunits, ASIC1 and ASIC2, to generate $H^+$-gated cation channels (8–10). ASIC3 shares the overall structure of DEG/ENaC proteins, including two transmembrane domains, a large extracellular loop containing 14 conserved cysteines, and intracellular N and C termini (11, 12). The intracellular domains of ASIC subunits might have several functions, including localizing the channels, controlling the number of channels on the cell surface, regulating channel function, and forming part of a scaffold important for the response to mechanical stimuli (11–13). Two proteins have been shown to interact with the intracellular domains of ASIC channels. The C termini of ASIC1 and ASIC2 share homology with type II PDZ (PSD-95, Drosophila disc-large protein, zonula occludens protein-1)-binding domains and bind PICK1 (protein interacting with C kinase-1) (14, 15). PICK1 may facilitate the interaction of ASIC2a with protein kinase C, and the interaction between ASIC1 and PICK1, regulated by phosphorylation, may provide a mechanism to control the cellular localization of ASIC1 (16, 17). The ASIC3 C terminus shares homology with type I PDZ-binding motifs. CIPP (channel-interacting PDZ domain protein), which contains four PDZ domains, is reported to interact with the ASIC3 C terminus and increase $H^+$-gated current (18).

The potential importance of protein-protein interactions for ASIC3 function led us to ask what proteins bind its intracellular domains. To identify interacting proteins, we used the yeast two-hybrid system and a candidate gene approach.

**EXPERIMENTAL PROCEDURES**

Yeast Two-hybrid Assay—Construction of the bait plasmid, hASIC3 (residues 476–531), in the GAL4(DB) vector pAS2-1 (Clontech) was described previously (14). This bait construct was transformed into the FJ622A yeast strain using the lithium acetate procedure and mated with yeast strain Y187 pretransformed with the human brain Matchmaker cDNA library (Clontech). The mRNA source was a normal, whole brain from a 37-year old Caucasian male as reported on the Clontech product analysis certificate. Mated cells were plated on Leu+, Trp+, His−.
plates supplemented with 5 mM 3-aminoo-1,2,4-triazole and grown for 10 days at 30 °C before positive clones were picked and streaked on plates lacking adenine, His, Leu, and Tpr. Library plasmids from clones that grew in the absence of adenine, His, Leu, and Tpr and that tested positive for β-galactosidase expression were isolated. They were co-transformed with either the bait vector or the original pAS2-1 vector into PFP9-2A to confirm the interaction. Those were specific for the bait were sequenced.

DNA Constructs—hASIC3 (residues 476–531), hASIC1c (residues 459–528), hASIC2 (residues 470–512), hp2NaC (residues 559–680), and hASIC6 (residues 559–610) were in the GAL4/DB vector pAS2-1 and the full-length mASIC3 construct in pMTr3 was purchased previously (14). The hASIC3 deletion constructs used for the yeast two-hybrid experiments were made by using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) for residues 476–527 and subcloning using PCR to amplify and insert the C-terminal deletions of hASIC3 residues 476–502, 476–513, and 513–531 into the unique EcoRI and BamHI sites of the GAL4/DB vector. Point mutations in the C terminus of the hASIC3 (residues 476–531) in pAS2-1 (see Fig. 1C) were made using the QuikChange site-directed mutagenesis kit. The mASIC3Δ4 (mASIC3 minus the C-terminal 4 amino acids) construct in pMTr3 was also made using the QuikChange mutagenesis kit. The GFP-tagged rat PSD-95 construct was a gift from Ian Macara. The rat ASIC3 cDNA (used in the electrophysiology studies) was cloned by reverse transcription (RT–PCR) of RNA isolated from Sprague-Dawley rat dorsal root ganglia using the 5′-primer 5′-CTCTGATGGATCCCTCCTGCTGTTGTTCTCAGG-3′ and the 3′-primer 5′-TACCCTGAGAGGCGGTTCG-3′. The PCR fragments were cloned into the pGEM-T easy vector (Promega). Point mutations in the C terminus of the hASIC3 were precipitated by adding anti-ASIC3, anti-mASIC3 (3), or anti-hASIC 6.4 affinity-purified antibodies (19) to 50 μg of tissue extracts in a final buffer of 25 mM HEPES, pH 7.4, 100 mM NaCl, 5 mM EGTA, 5 mM EDTA, 1 mM dithiothreitol, 100 μM aprotinin, 20 μM leupeptin, 10 μM pepstatin A and rocking at 4 °C. Protein A-Sepha- sorose was washed three times with 1% Triton X-100, 0.5% sodium deoxycholate, 1% SDS, 0.4 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, 20 μg/ml leupeptin, 10 μg/ml pepstatin A and rocked at 4 °C. The blots were washed five times with TBS-buffered solution, incubated with horseradish peroxidase-conjugated secondary antibody (1:5000) (Amersham Biosciences) or horse-radish peroxidase-linked Protein A 1:10,000 (Amersham Biosciences). Proteins were detected by enhanced chemiluminescence (Pierce).

Immunofluorescence—COS-7 cells were grown on chamber slides coated with collagen. Cells were fixed with 4% formaldehyde in phosphate-buffered saline, permeabilized with 0.1% Triton X-100 in phosphate-buffered saline, blocked with SuperBlock (Pierce), and incubated with primary antibodies anti-mASIC3 serum (1:750) and anti-PSD-95 (1:1500), anti-Myc (1:1000), or anti-HA (1:1250); guinea pig anti-ASIC3 (1:8000) and anti-Myc (1:1000) followed by the secondary antibodies goat anti-rabbit Alexa 488 (1:1250) and goat anti-mouse Alexa 488 (1:1250); or goat anti-guinea pig Alexa 568 (1:1250) and goat anti-mouse Alexa 568 (1:1250) and goat anti-mouse Alexa 568 (1:1250). The cycling parameters using the RoboCycler thermocycler (Stratagene) consisted of 40 cycles of 94 °C for 45 s, 60 °C for 45 s, and 72 °C for 2 min.

Immunoprecipitation from Tissue—Rat tissues (brain, spinal cord, and dorsal root ganglia) were isolated and frozen at −80 °C. Lysates were prepared by homogenizing the tissue匀 before homogenization, or by using whole tissue匀. Immunoprecipitation from Tissue匀 was precipitated by adding anti-PSD-95, anti-mASIC3 (3), or anti-hASIC 6.4 affinity-purified antibodies (19) to 50 μg of tissue extracts in a final buffer of 25 mM HEPES, pH 7.4, 100 mM NaCl, 5 mM EGTA, 5 mM EDTA, 1 mM dithiothreitol, 100 μM aprotinin, 20 μM leupeptin, 10 μM pepstatin A and rocking at 4 °C. Protein A-Sepharose was washed three times with 1% Triton X-100, 0.5% sodium deoxycholate, 1% SDS, 0.4 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, 20 μg/ml leupeptin, 10 μM pepstatin A and rocked at 4 °C. The blots were washed five times with TBS-buffered solution, incubated with horseradish peroxidase-conjugated secondary antibody (1:5000) (Amersham Biosciences), and blocked with 5% bovine serum albumin and incubated with anti-PSD-95 (1:1000), anti-mASIC3 (1:250), or anti-hASIC 6.4 (1:1000) affinity-purified antibody for 2 h. The blots were washed five times with TBS-buffered solution, incubated with horseradish peroxidase-conjugated protein A 1:10,000, washed with 5% Tween 20 in TBS, and developed with the enhanced chemiluminescence reagent (Pierce).

Electrophysiology—Whole-cell patch clamp recordings (at −70 mV) from CHO cells were performed with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) and acquired and analyzed with Pulse/Pulse-8.30 (HEKA Electronics, Lambrecht, Germany) and Igor Pro 3.1.6 (WaveMetrics, Lake Oswego, OR) software. Experiments were performed at room temperature. Currents were filtered at 5 kHz and sampled at 2 or 0.2 kHz. Series resistance was compensated by at least 50%. Micropipettes (2–5 megaohms) were filled with internal solution: 100 mM KCl, 10 mM EGTA, 40 mM HEPES, and 5 mM MgCl2, pH 7.4 with KOH. External solution contained: 120 mM NaCl, 5 mM CaCl2, 10 mM EGTA, 40 mM HEPES, and 5 mM MgCl2, pH 7.4 with KOH. External solution contained: 120 mM NaCl, 5 mM CaCl2, 10 mM EGTA, 40 mM HEPES, and 5 mM MgCl2, pH 7.4 with KOH. External solution contained: 120 mM NaCl, 5 mM CaCl2, 10 mM EGTA, 40 mM HEPES, and 5 mM MgCl2, pH 7.4 with KOH. Surface Biotinylation—Surface proteins on 48-h post-transfected CHO cells were biotinylated as described previously (20). After labeling, cells were washed twice with TBS and lysed in lysis buffer (25 mM HEPES, pH 7.4, 100 mM NaCl, 5 mM EGTA, 5 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 1% SDS, 0.4 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, 20 μg/ml leupeptin, 10 μg/ml pepstatin A). Cell surface proteins were isolated by incubating
RESULTS

Identification of MAGI-1b, Lin-7b, and PIST with a Yeast Two-hybrid Screen—To find proteins that interact with ASIC3, we used its intracellular C terminus as the bait to screen a human brain cDNA library in the yeast two-hybrid system. We identified three clones specific for the bait: MAGI-1b, Lin-7b, and PIST. MAGI-1b is a member of a family of MAGI proteins, and several different splice variants of MAGI-1 have been identified, including MAGI-1a, -1b, and -1c (22, 23).

Lin-7b, also named MALS (mammalian Lin-7 protein) or Veli (vertebrate LIN-7 homologs) contains an N-terminal domain that binds CASK and a PDZ domain at its C terminus (24–26). Both Lin-7b and MAGI isoforms are thought to provide a scaffold linking receptors and channels with cytoskeletal proteins and enzymes at sites of cell-cell contact such as tight junctions in epithelial cells and synapses in neurons.

PIST is an intracellular protein first identified by its interaction with TC10, a Rho GTPase (27). PIST contains two coiled-coil domains, a leucine zipper domain embedded in the second coiled-coil domain, and a PDZ domain at its C terminus. Splice variants of PIST, such as CAL (CFTR-associated ligand) and FIG (fused in glioblastoma), are thought to participate in the trafficking of proteins out of the trans-Golgi network and retention of membrane proteins inside the cell (28, 29).

The interaction of MAGI-1b, Lin-7b, and PIST was specific for the ASIC3 C terminus; the C termini of ASIC1, ASIC2, αENaC, and βENaC failed to interact with these proteins (Fig. 1A). Deleting the distal half of the ASIC3 C terminus eliminated the interaction with all three proteins, whereas deleting the last 4 or 8 C-terminal residues also abolished the interaction. These results suggested that ASIC3 interacts with the three PDZ domains via a PDZ-binding motif at its C terminus. The C-terminal 4 residues of hASIC3 (VTQL) correspond to a type I PDZ-binding motif (XX/S/T)(X) where X is any amino acid) (30). Mutating the Leu at the 0 position to Ala abolished the interaction with MAGI-1b, Lin-7b, and PIST (Fig. 1C). In addition, mutating the Thr at the –2 position abolished the interaction with MAGI-1b and Lin-7b (PIST was not tested). In contrast, mutating the –3 Val had no effect on the interactions. Mutation of the –1 Gin did not alter the interaction with MAGI-1b or Lin-7b but disrupted the interaction with PIST. Thus, binding conforms to an interaction between the ASIC3 C terminus and PDZ domains in MAGI-1b, Lin-7b, and PIST most likely through their PDZ domains.

ASIC3 Interacts with MAGI-1b, Lin-7b, and PIST in COS-7 Cells—To test the interaction between ASIC3 and these proteins, we transfected COS-7 cells with mouse ASIC3 and epitope-tagged MAGI-1b, Lin-7b, and PIST. Immunoprecipitation of ASIC3 co-precipitated each of these PDZ proteins (Fig. 2). We used PIST to test the importance of the C-terminal 4 residues in the interaction with PDZ domain-containing proteins. Deleting the last 4 residues of ASIC3 (ASIC3-4) prevented co-precipitation of PIST.

PSD-95 Interacts with ASIC3 and Requires Its PDZ-binding Domain—In addition to their interaction with ASIC3, previous studies showed that the PDZ domain-containing proteins MAGI-2 (the major neuronal MAGI protein) and Lin-7 interact with the N-methyl-D-aspartate receptor subunits (26, 31). Previous studies showed that the PDZ domain scaffold protein PSD-95 also interacts with NR2 subunits (32). PSD-95 contains a Src homology 3 domain, a guanylate kinase domain, and three N-terminal PDZ domains (33). In addition, both MAGI-2 and PSD-95 interacted with the shaker type K+ channels and the neuronal cell surface molecule neuroligin (31, 34, 35). Therefore, we hypothesized that ASIC3 may bind PSD-95 through its PDZ-binding domain.

Coexpressing ASIC3 Alters the Cellular Distribution of PSD-95 and PIST—Previous studies have shown that coexpressing a
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PDZ domain protein and its interacting partner in some cases alters the cellular distribution of one or both proteins. The distribution often assumes a clustered pattern with protein-protein binding at intracellular sites. This appearance indicates an interaction between the two proteins but does not necessarily indicate an altered distribution at the cell surface. For example, PSD-95 expression caused clustering of shaker type K^+/H11001 channels (34), and MAGI-2 clustered the N-methyl-D-aspartate receptor NR2A in transfected COS-7 cells (36). To determine whether Lin-7b, PIST, MAGI-1b, or PSD-95 alters ASIC3 distribution, we expressed these proteins in COS-7 cells. When expressed alone, all four PDZ domain-containing proteins showed a diffuse staining pattern throughout the cell, whereas ASIC3 showed a reticular pattern (Fig. 4A). Coexpressing ASIC3 and PSD-95 altered the distribution of both proteins so that they colocalized in small clusters (Fig. 4B). In addition, coexpressing ASIC3 changed the distribution of PIST so that it overlapped the reticular expression pattern of ASIC3. In contrast, neither ASIC3 nor Lin-7b (Fig. 4B) or MAGI-1b (not shown) changed their distribution when coexpressed. Deleting 4 residues from the ASIC3 C terminus prevented the co-localization of ASIC3 and PSD-95 and prevented the redistribution of PIST (Fig. 4B). Taken together, the yeast two-hybrid data, the co-immunoprecipitation results, and the colocalization indicate that the ASIC3 PDZ-binding motif interacts with the PDZ domains of PSD-95, Lin-7b, PIST, and MAGI-1b. However, the colocalization results also suggested the potential for different functional consequences when the proteins interact.

ASIC3 and Interacting Proteins Are Expressed in Spinal Cord and DRG—Expression of Lin-7b, PIST, MAGI-1b, and PSD-95 was reported in the central nervous system (22, 24–27, 33), whereas ASIC3 expression has been described primarily in peripheral neurons (1–3). To test whether expression occurs in the same tissue, we used RT-PCR to detect their transcripts. We found Lin-7b, PSD-95, MAGI-1b, and PIST mRNA present in DRG, spinal cord, and brain (Fig. 5). ASIC3 transcripts were also detected in all three tissues. Although we detected PSD-95 transcripts in DRG, previous studies reported PSD-95 in brain and spinal cord but not DRG (37). To test whether ASIC3 protein was present outside the peripheral nervous system and found it in the spinal cord (Fig. 6B). Although the ability of commercially available antibodies to detect the other PDZ domain proteins limited additional studies, the data indicate that ASIC3 and PSD-95 are both present in the spinal cord and DRG where they might interact. The presence of transcripts for Lin-7b, MAGI-1b, and PIST suggest that they might also be present at the same site as ASIC3.

To test for an interaction between ASIC3 and PSD-95 in vivo, we immunoprecipitated PSD-95 from spinal cord and found...
that it co-precipitated ASIC3 (Fig. 6C). In contrast, PSD-95 did not co-precipitate with ASIC1, which is also expressed in spinal cord (38).

**PSD-95 and Lin-7b Alter ASIC3 $\text{H}^+$-gated Currents**—The interaction of these proteins with ASIC3 suggested they might alter its current. Application of a pH 5 solution to CHO cells expressing ASIC3 generated transient inward currents (Fig. 7A), consistent with previous reports (1, 2). Coexpression of rat ASIC3 with PSD-95 reduced the amplitude of acid-evoked currents ~5-fold (Fig. 7, A and B). When we coexpressed ASIC3 with Lin-7b, we obtained the opposite result; current density increased ~8-fold. The PSD-95- and Lin-7b-induced alterations in current amplitude occurred without major changes in the rate of current desensitization or the sensitivity to pH (Fig. 7, C and D). Both MAGI-1b and PIST altered current with an increase of ~2.5- and ~2.1-fold increase over control, respectively, with no change in channel properties. Because PSD-95 and Lin-7b had the largest and opposite effects on ASIC3 current, we studied their effects on ASIC3 in more detail.

To learn whether the functional effects arose from an interaction of the ASIC3 PDZ-binding motif with PSD-95 and Lin-7b, we repeated the experiments with rat ASIC3 missing its 4 C-terminal residues (ASIC3Δ4). When expressed alone, ASIC3 and ASIC3Δ4 generated $\text{H}^+$-gated currents of similar amplitude, but without the C-terminal residues the effects of PSD-95 and Lin-7b were blunted (Fig. 7B).

The results indicate that the PDZ-binding motif of ASIC3 is required for the interaction with PSD-95 and Lin-7b. As an additional test of specificity, we studied ASIC1a; PSD-95 had no effect, while Lin-7b showed a small increase in current (Fig. 7E). To test whether the C-terminal motif of ASIC3 is sufficient for an effect on function, we substituted the 4 C-terminal amino acids mutated to VTRL (ASIC1VTRL) with either PSD-95 or Lin-7b. n = 8–16; *, p < 0.05 compared with ASIC3Δ4; †, p < 0.01 compared with ASIC3; ‡, p < 0.05 compared with ASIC3 + Lin-7b. C, time constants of desensitization as measured from single exponential fits to the falling phase of the currents evoked by pH 6 application. n = 8–16. D, relationship between pH and current. Currents were normalized to those evoked by pH 5. n = 3 for all points. E, peak current densities evoked by pH 5 application to CHO cells coexpressing ASIC1a or ASIC1a with the terminal 4 amino acids mutated to VTRL (ASIC1VTRL) with either PSD-95 or Lin-7b. n = 8–16; *, p < 0.05 compared with ASIC1a; †, p < 0.05 compared with ASIC1a + Lin-7b. pF, picofarads.
Our results demonstrate that ASIC3 binds the PDZ domain-containing proteins PSD-95, Lin-7b, MAGI-1b, and PIST. These interactions occurred through a PDZ-binding motif at the C terminus of ASIC3 as shown by the yeast two-hybrid assay and co-immunoprecipitation in COS-7 cells. In addition, for some of the interactions, coexpression altered the pattern of immunostaining in COS-7 cells. Moreover we found that PSD-95 coimmunoprecipitated ASIC3 from spinal cord. However, the interactions differed for the various PDZ-containing proteins; the most striking difference occurred when ASIC3 was coexpressed with PSD-95 and Lin-7b.

PSD-95 reduced ASIC3 acid-evoked currents. Current fell because PSD-95 decreased the amount of ASIC3 in the cell membrane. ASIC3 currents measured in the presence of PSD-95 retained the acid sensitivity and desensitization rate of ASIC3 expressed alone, consistent with a reduction in the number of cell surface channels rather than an alteration of their properties. The finding that PSD-95 did not reduce the total amount of ASIC3 in the cell suggested that it did not decrease cell surface protein by stimulating ASIC3 degradation. Although Lin-7b also bound the C terminus of ASIC3, it had an effect opposite from that of PSD-95. Lin-7b increased ASIC3 current by increasing ASIC3 cell surface expression. Like PSD-95, we found no evidence that the interaction altered the properties of ASIC3 currents. Consistent with our findings, recent studies have shown that Lin-7b interacted with and retained the epithelial γ-aminobutyric acid transporter (BGT-1) at the membrane of Madin-Darby canine kidney cells (39). Lin-7b also stabilized the inward rectifying K+ channel Kir 2.3 at the plasma membrane, thereby increasing channel current (40). The functional expression of Xenopus ENaC, a member of the DEG/ENaC family, also requires its association with Apx (apical protein Xenopus) and α-spectrin, which may sequester and stabilize ENaC at the cell surface (41). Interactions with more than one protein containing a PDZ domain have also been observed to regulate CFTR; PIST (CAL) reduces and the Na+/H+ exchanger-regulatory factor increases CFTR expression on the cell surface (28). A recent report indicated that CIPP also increased ASIC3 currents (18). Although the mechanism was not investigated, like Lin-7b, CIPP did not alter the properties of ASIC3 currents. Thus, we speculate that as with Lin-7b, CIPP may also increase the amount of ASIC3 in the plasma membrane.

Although we found that four different proteins (PSD-95, Lin-7b, MAGI-1b, and PIST) interacted with the ASIC3 C terminus, there were differences. For example, in the yeast two-hybrid assay the Arg at position −1 in ASIC3 was important for the interaction with PIST but not MAGI-1b or Lin-7b. In addition, while the ASIC3 PDZ-binding motif was required for the functional effects of both Lin-7b and PSD-95, incorporating the ASIC3 C terminus on ASIC1a reconstituted the stimulatory effect of Lin-7b but not the current reduction produced by PSD-95. Thus additional sequences in ASIC3 appear to be necessary for PSD-95 to interact with ASIC3; similar conclusions have been made for the interaction between other PDZ domain proteins and their partners (42, 43).

The findings suggest the possibility of substantial complexity in the interactions of ASIC3 with a cytoplasmic scaffold built from multiple components. Although we show an in vivo interaction with PSD-95, ASIC3 may interact with some or all of the other proteins identified here as well as with CIPP (18). As with other DEG/ENaC channels (10, 44), several ASIC subunits multimerize to form a functional channel, providing a single channel complex with the opportunity for multiple interactions. Conversely several of the interacting proteins have more than one PDZ domain (MAGI-1b has five, CIPP has four, and PSD-95 has three), suggesting they could bind more than one of the C termini in an ASIC3 channel or other receptors and channels at the same time. In addition, because ASIC3 hetero-multimerizes with other ASIC subunits, ASIC3 might be drawn indirectly into interactions with other proteins such as PICK1, which binds ASIC1 and ASIC2 (14, 15). Moreover the PDZ-containing proteins we identified could generate additional interactions because they also contain other protein-binding domains, including leucine zipper, Src homology 3, WW, guanylate kinase, and coiled-coil domains.

In addition to its previously demonstrated importance in peripheral neurons, our data identify ASIC3 in the spinal cord where it interacts with PSD-95. Interestingly spinal cord PSD-95 also interacts with the N-methyl-D-aspartate receptor NR2A/B (37); both ASIC3 and NR2 subunits also bind Lin-7b and MAGI-2 (26, 31, 36). The presence of ASIC3, NR2 subunits, and this scaffolding complex in the spinal cord raises questions about
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ASIC3 function at that site. Recent studies in PSD-95 mutant mice and mice injected with PSD-95 antisense oligonucleotides have shown a reduction in the neuropathic reflex sensitization that develops upon thermal and mechanical hyperalgesia (37, 45, 46). In addition, mice lacking ASIC3 failed to develop mechanical hyperalgesia and central sensitization that normally follow repeated intramuscular acid (5). Our data combined with earlier studies suggest that this sensitization might be due to signaling mechanisms in either peripheral or spinal cord neurons. In either case, they suggest that ASIC3 may be a target for the development of agents targeting nociception.

These findings suggest that ASIC3 interacts with a complex intracellular protein scaffold. These interactions could influence channel function in a number of ways. They might modulate nociceptive and mechanosensory functions by controlling the amount of cell surface protein and hence total ASIC3 current. They might also influence ASIC3 localization. Because the contribution of DEG/ENaC channels, including ASIC3, to mechanosensation may involve a tethered channel mechanism, interactions with an intracellular scaffold may also be a key component of the mechanosensory process.


REFERENCES