Annexin II Light Chain p11 Promotes Functional Expression of Acid-sensing Ion Channel ASIC1a*

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Acid-sensing ion channels (ASICs) have been implicated in a wide variety of physiological functions. We have used a rat dorsal root ganglion cDNA library in a yeast two-hybrid assay to identify sensory neuron proteins that interact with ASICs. We found that annexin II light chain p11 physically interacts with the N terminus of ASIC1a, but not other ASIC isoforms. Immunoprecipitation studies confirmed an interaction between p11 and ASIC1 in rat dorsal root ganglion neurons in vivo. Coexpression of p11 and ASIC1a in CHO-K1 cells led to a 2-fold increase in expression of the ion channel at the cell membrane as determined by membrane-associated immunoreactivity and cell-surface biotinylation. Consistent with these findings, peak ASIC1a currents in transfected CHO-K1 cells were up-regulated 2-fold in the presence of p11, whereas ASIC3-mediated currents were unaffected by p11 expression. Neither the pH dependence of activation nor the rates of desensitization were altered by p11, suggesting that its primary role in regulating ASIC1a activity is to enhance cell-surface expression of ASIC1a. These data demonstrate that p11, already known to traffic members of the voltage-gated sodium and potassium channel families as well as transient receptor potential and chloride channels, also plays a selective role in enhancing ASIC1a functional expression.

Acid-sensing ion channels (ASICs) are voltage-independent H+-gated ion channels belonging to the amiloride-sensitive degenerin/epithelial sodium channel superfamily of receptor channels. ASICs have been implicated in a number of different physiological processes such as nociception (1, 2); mechanosensation (3–5); learning, memory, and fear behavior (6–8); sour taste (9); visual transduction (10); and cochlear function (11). Four genes encoding six different ASIC subunits (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4) have been cloned so far. Each ASIC subunit contains intracellular N- and C-termini, two transmembrane domains, and a large extracellular loop with cysteine-rich regions. The ASIC subunits can assemble to form functional homo- or heteromultimers that are mainly permeable to Na+. ASIC1a (also known as brain sodium channel-2) was cloned from human and rat brain cDNA libraries (12, 13). It is primarily expressed in the brain and somatic afferent neurons of the dorsal root (DRG) and nodose ganglia as well as in spinal and vagal visceral afferents. In DRGs, ASIC1a is expressed mainly in the small diameter nociceptive neurons, where it senses any acidification of the external milieu occurring during various pathophysiological and painful states such as inflammation, hematomas, edema, fractures, blisters, infection, ischemia, and the presence of tumors (14). ASIC1a is also involved in mechanosensory function when expressed in large DRG fibers innervating the colon and in vagal afferents innervating the stomach and the esophagus (5). In the brain, ASIC1a is the main H+-gated current and was shown to be involved in synaptic plasticity and fear conditioning (6–8). In DRG and brain neurons, ASIC1a is often associated with the ASIC2a subunit to form heteromultimeric channels (15), but it also exists in the form of functional homomultimeric channels that can be selectively blocked by the tarantula venom psalmotoxin-1 (16). Unlike the other ASICs, ASIC1a was found to be permeable to Ca2+ as well as Na+, with a Na+/Ca2+ permeability ratio of 2.5 (13). Several studies confirmed this finding (17–19), but others found a lower Ca2+ permeability for ASIC1a (20, 21). Ca2+ imaging in COS-7 cells transiently expressing ASIC1a recently demonstrated that homomeric ASIC1a channels are a major non-voltage-gated pathway for Ca2+ entry in cells (22). It was also demonstrated that Ca2+ overload through ASIC1a channels makes a major contribution to hippocampal neuron damage in strokes. A role for ASIC1a in Ca2+ overload during brain ischemia is consistent with the fact that lactic acid, an enhancer of ASIC currents (23), and protons are both produced (by anaerobic glycolysis and ATP hydrolysis, respectively) during ischemia. In fact, it appears that the main source of Ca2+ entry occurring during ischemia is not through ionotropic glutamate receptors, but through ASIC1a channels, as ASIC1a blockade is a more efficient way to limit ischemic damage than the use of glutamate antagonists (24). A role for ASIC1a activation in cell damage is in line with the known involvement of Caenorhabditis elegans degenerin/epithelial sodium channel gain-of-function mutants that cause cell degeneration (25). Both native ASIC1a and degenerin mutants cause cell damage by increasing Ca2+ uptake. The neurotoxicity of MEC-4(d), a C. elegans hyperactive mutant channel of the degenerin family, is a consequence of enhanced Ca2+ permeability (26). Thus, apart from its acid-sensing function in various tissues, ASIC1a activation may be a major factor in the biological events that lead to neuronal damage. Therefore, the search for factors regulating ASIC1a function has become more pressing with respect to the development of selective pharmacological agents. Intracellular regulation of ASIC function must occur via the short intracellular N- and C-terminal domains of these channels. Previous studies (30, 31, 35, 36) have identified possible interacting partners for ASICs targeted to the C-terminal tail. Here, using a yeast two-hybrid assay, we have trapped proteins interacting with the N-terminal domain of rat (r) ASIC1a. We report that p11, a member of the S100 small phospholipid- and Ca2+-binding protein family, binds to ASIC1a and is

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2 The abbreviations used are: ASIC, acid-sensing ion channel; DRG, dorsal root ganglion; r, rat; GFP, green fluorescent protein; Raf, rafinose; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; GST, glutathione S-transferase; PBS, phosphate-buffered saline; TRITC, tetramethylrhodamine isothiocyanate; MES, 4-morpholineethanesulfonic acid.
part of the intracellular machinery that delivers the channel to the cell surface.

EXPERIMENTAL PROCEDURES

Constructs—The N-terminal regions of rASIC1a, rASIC2a, rASIC3, and rASIC4 (corresponding to residues 1–43, 1–42, 1–43, and 1–67, respectively) were amplified by PCR either from a rat DRG cDNA library (kindly provided by Dr. Moses V. Chao, New York University) (ASIC1a, ASIC3, and ASIC4) or from cDNA prepared from rat DRGs (ASIC2a). The PCR fragments were inserted into the yeast pEG202 vector downstream of the full-length LexA coding region at the EcoRI and XhoI restriction sites. In the DRG cDNA library, cDNAs were cloned into pJG4-5, where their transcription is under the control of the GAL1 promoter.

A sequence corresponding to the FLAG epitope (GATTACAAG-GATGACGACGATAAG) and a NotI site were introduced by PCR after nucleotide 444 of rASIC1a, and the resulting rASIC1a-FLAG protein was subcloned into pRK7. This construct was used to study the surface expression of the ion channel in cell lines.

In all electrophysiological studies, we used full-length rASIC1a and rASIC3 cDNAs subcloned into the baculovirus vector pl8RS2-DsRed-Express (BD Biosciences). Rat full-length p11 cDNA was inserted into pBS500 downstream of the green fluorescent protein (GFP) sequence as described (27) for expression in mammalian cells and into the pQE30 vector (Qiagen Inc.) downstream of the Hisi sequence for bacterial expression.

Yeast Two-hybrid System—Yeast strain EGY48 (ura3, his3, trp1, leu2) was transformed consecutively with the reporter plasmid pSH18-34 (lacZ), the bait pEG202-ASIC1a-NT (where "NT" is the N-terminal region), and the cDNA library using the lithium acetate method. Approximately $1 \times 10^7$ transformants were screened, and clones interacting with the bait were selected on medium containing galactose (Gal/Raf/Ura/His/Trp/Leu) after 2–3 days of incubation at 30°C. These clones were then plated onto medium containing glucose and lacking uracil, histidine, tryptophan, and leucine (Gal/Raf/Ura/His/Trp/Leu) after 2–3 days of incubation at 30°C. These clones were then plated onto medium containing glucose and lacking uracil, histidine, and tryptophan (Glc/Ura/His/Trp) to turn off the GAL1 promoter and to stop the expression of the DRG cDNA in pJG4-5 and grown for 1 day at 30°C. Replicas were then made on the following plates: Glc/Ura/His/Trp/X-gal and Gal/Raf/Ura/His/Trp/X-gal to test for $\beta$-galactosidase activity and Glc/Ura/His/Trp/X-gal to test for glutathione S-transferase (GST) fusion protein. GST was induced for 1 h at 30°C and the results were analyzed after 1, 2, and 3 days. Only colonies that grew on galactose media lacking leucine but not on glucose media lacking leucine and that turned blue on galactose/X-gal plates but remained white on glucose/X-gal plates were considered as positive. Of 51 positive clones, 14 encoding full-length p11 were identified as interacting with rASIC1a.

Glutathione S-Transferase (GST) Pull-down Assay—The rASIC1a and rASIC3 N-terminal domains were subcloned into pGEX-5X-1 (Amersham Biosciences) and expressed as GST fusion proteins in Escherichia coli BL21. The original pGEX-5X-1 vector was used to produce GST alone. The bacteria were resuspended in ice-cold binding buffer (phosphate-buffered saline (PBS) containing 1% Triton X-100; apro- nin, leupeptin, pepstatin A (each 1 µg/ml), benzamidine (250 µg/ml), iodoacetamide (370 µg/ml), and 0.5 µM phenylmethylsulfonyl fluoride) and sonicated three times for 20 s. After centrifugation at 4100 rpm for 10 min, the supernatant was incubated for 30 min at room temperature with glutathione-Sepharose 4B (Amersham Biosciences), and the beads were washed three times with ice-cold PBS and resuspended in binding buffer. Full-length p11 cDNA was subcloned into pQE30 (Qiagen QIAexpressionist system) and expressed as a His-tagged protein in E. coli M15(pREP4). Bacteria were lysed as described above, and the crude supernatant was used in the pull-down assay without further purification. GST- and GST fusion protein-bound glutathione-Sepharose beads were incubated with Hisp-p11 for 1 h at 30 min at room temperature with mild agitation. The samples were then washed four times with ice-cold PBS, resuspended in Laemmli SDS sample buffer, heated for 5 min at 95°C, and run on two SDS-polyacrylamide gels (10 and 14%). One of the gels (10%) was stained with Coomassie Blue, and the other one (14%) was used for Western blotting. The proteins were transferred to nitrocellulose membrane (Hybond, Amersham Biosciences). The membrane was blocked with 5% nonfat milk in PBS containing 0.1% Tween and incubated with rabbit anti-His, antibody (Camlab) diluted 1:1000 in the blocking buffer overnight at 4°C. The membrane was then washed three times with PBS containing 0.1% Tween, and the secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit IgG; Jackson ImmunoResearch Laboratories, Inc.) was applied at 1:1000 dilution in PBS containing 0.1% Tween for 2 h at room temperature. His-tagged p11 was detected using ECL detection reagent (Amersham Biosciences). Bands intensities were compared using ImageJ software (available at rsb.info.nih.gov/ij/), which calculates pixel value statistics of user-defined areas.

Co-immunoprecipitation—DRGs were extracted from four wild-type and four ASIC1 knock-out mice, homogenized in lysis buffer (50 mM Tris- HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.1% SDS, 1 µg/ml aprotinin, 1 µg/ml leupeptin, 1 µg/ml pepstatin A, 250 µg/ml benzamidine, 370 µg/ml iodoacetamide, and 0.5 µM phenylmethylsulfonyl fluoride), and incubated on ice for 1 h. After centrifugation at 14,000 rpm for 2 min, the supernatant was precleared by addition of 50 µl of protein A-Sepharose beads (Amersham Biosciences) and incubation on ice for 1 h. This was carried out twice, and after centrifugation at 10,000 rpm for 1 min, the precleared supernatant was retained and kept on ice. Protein A-Sepharose beads were loaded with rabbit anti-chicken IgY (Sigma) for 1 h at room temperature and washed three times with Tris-buffered saline. Subsequently, chicken anti-p11 IgY antibody was added to the beads and incubated for 1 h at room temperature. After washing three times with Tris-buffered saline, the precleared DRG lysate was mixed with the antibody-loaded beads and incubated overnight at 4°C. The beads were washed three times with lysis buffer, and all supernatant was removed. They were resuspended in Laemmli SDS sample buffer, heated for 5 min at 95°C, and run on a 7% SDS-polyacrylamide gel. Western blotting was carried out as described above using rabbit anti-ASIC1 antibody (Alomone Labs) at 1:200 dilution and horseradish peroxidase-conjugated goat anti-rabbit IgG antibody at 1:1000 dilution.

Immunocytochemistry—CHO-K1 cells were cultured on 13-mm poly-L-lysine-coated coverslips and transfected with 0.2 µg of pRK7-rASIC1a-FLAG and 0.8 µg of either pBS500-p11 or pBS500 (as a negative control) using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. 24 h post-transfection, cells were fixed with 4% paraformaldehyde for 5 min at room temperature, permeabilized if appropriate with 0.1% Triton X-100 in blocking solution (10% goat serum in PBS) for 15 min at room temperature, and incubated for 2 h at room temperature with rabbit anti-FLAG antibody (Sigma) diluted 1:2000 in blocking solution with or without Triton X-100. The cells were washed three times with PBS, incubated with goat anti-rabbit TRITC antibody (Jackson ImmunoResearch Laboratories, Inc.) at 1:200 dilution for 1 h at room temperature, and washed three times with PBS before the coverslips were mounted onto slides.
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Biotinylation of Cell Surface Proteins—CHO-K1 cells were cultured in 10-cm dishes and transfected with 1.5 μg of pRK7-rASIC1a-FLAG and 3.5 μg of either pBS500-p11 or pBS500 (as a negative control) using Lipofectamine 2000 according to the manufacturer’s instructions. 1 day after transfection, biotinylation and recovery of plasma membrane proteins were carried out using the Pierce cell-surface protein biotinylation and purification kit according to the manufacturer’s protocol. Samples were analyzed by immunoblotting using rabbit anti-FLAG antibody at 1:2000 dilution. Bands intensities were compared using ImageJ software.

Whole Cell Voltage Clamp Experiments—CHO-K1 cells were transfected with equimolar amounts of ASIC1a and p11-GFP or with GFP as a negative control and used for electrophysiology 24 h after incubation. Successfully cotransfected cells were identified using an epifluorescence inverted microscope. The H+-gated ASIC currents were recorded using an Axopatch 200B amplifier (Axon Instruments, Inc.). Pipettes were pulled from borosilicate glass capillaries with a P-97 puller (Sutter Instrument Co.) and had resistances of 1–3 MΩ. Currents were digitized with a Digidata 1322A data acquisition system (Axon Instruments, Inc.). Data were recorded and stored using Clampex 8.1 (Axon Instruments, Inc.). Currents were low pass-filtered at 2 kHz and sampled at 11 kHz. Capacity transients were cancelled, and series resistance was compensated by 80% minimum. Voltages were not corrected for liquid junction potentials. Recordings were performed at room temperature. Off-line analysis and fits were performed using Clampfit 9.0 (Axon Instruments, Inc.) and SigmaPlot 8 (Systat Software Inc.). pH-current density relationships were fitted with a Hill equation of the following form: \( I(H^+) = I_{\text{max}} (H^+)^{(K + [H^+]^{-n})} \), where \( I_{\text{max}} \) is the maximal ASIC1a current density at a given holding potential, \( [H^+] \) is the concentration of protons, \( n \) is the Hill coefficient that estimates the degree of cooperativity in ligand binding, and \( K \) is the pH value that produces a current density that is 50% of \( I_{\text{max}} \). The time constants of desensitization of ASIC currents were derived from single exponential fits of the decaying phase of the currents. Values are expressed as means ± S.E. The statistical significance of differences between sets of data was tested using Student’s t test. The pipette solution contained 126 mM CsCl, 14 mM CsF, 1.2 mM MgCl₂, 1.2 mM CaCl₂, 3 mM Na₂EGTA, 4 mM MgATP, and 10 mM HEPES (pH 7.3). The bath solution contained 140 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, and 5 × 10⁻² mM capsaicine (pH 7.4). For acidic solutions, HEPES was replaced with MES. CHO-K1 cells do not express an endogenous ASIC current. Nevertheless, capsaicine was added to extracellular solutions because CHO-K1 cells expressed a small endogenous capsaicin-insensitive slowly desensitizing H⁺-gated current that was blocked by capsaizpine (data not shown). Rapid changes in extracellular pH were achieved in 20 ms by a RSC-160 rapid solution changer (Bio-Logic Science Instruments) commanding a 6-outlet microperfuson system.

RESULTS

p11 Interacts with ASIC1a in the Yeast Two-hybrid Assay—To study the regulation of ASIC1a, we used a yeast two-hybrid system and screened a rat DRG cDNA library to find proteins interacting with the intracellular N-terminal domain of this ion channel. From ~1–2 × 10⁷ yeast transformants, 51 interacting clones were selected. Of these, 14 clones encoding the full-length p11 protein were identified. To check the specificity of the interaction between p11 and the ASIC1a N terminus, yeast strain EGY48 containing the reporter plasmid pSH18-34 was sequentially transformed with plasmids encoding the N-terminal domain of rASIC2a, rASIC3, or rASIC4 and p11 and plated onto Gal/

![FIGURE 1. p11 Interacts with ASIC1a. A, the yeast two-hybrid system uses the reporter gene LEU2 such that only yeast expressing a protein that interacts with the bait can grow on media lacking leucine. Three transformants (lanes 1–3) of each condition were grown in liquid Glc/Ura ‘His’/ ‘Trp’ medium until they all reached A₁₁ = 1, and 10-μl drops of 10⁻² and 10⁻³ dilutions of the cultures were plated on Gal/Raf/Ura ‘His’/ ‘Trp’/ ‘Leu’ medium. B, ASIC1a-NT-GST can pull down His-tagged p11. Pull-down products were analyzed by SDS-PAGE, followed by Coomassie Blue staining (upper panel) and Western blotting using anti-His antibody (lower panel). GST was used as a negative control. C, ASIC1 and p11 interact in mouse DRGs. Anti-p11 antibody was used to pull down p11 and its interacting partners from wild-type (WT) and ASIC1 knock-out (KO) mouse DRGs; the Western blot was probed with anti-ASIC1 antibody. Rabbit IgG was used in the co-immunoprecipitation and was detected on the immunoblot by horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (heavy chain of ~55 kDa).](https://example.com/figure1.png)
p11 compared with the control (Fig. 2). The localization of ASIC3 did not appear to be affected by coexpression of p11 (data not shown).

To confirm the results obtained by immunocytochemistry, surface proteins of CHO-K1 cells coexpressing rASIC1a-FLAG and either p11-GFP or GFP were biotinylated and purified with streptavidin-conjugated agarose beads. Samples were analyzed by immunoblotting using anti-FLAG antibody (Fig. 3). Consistent with the results described above, we observed that the fraction of rASIC1a-FLAG protein located at the cell surface increased in the presence of p11. As determined using ImageJ software, the intensity of the band corresponding to cell-surface ASIC1a in the presence of p11 was ∼2.5 times higher compared with the control.

**Electrophysiology**—To examine any functional effect of p11 on ASIC1a, whole cell patch clamp experiments were carried out in CHO-K1 cells cotransfected with ASIC1a and p11-GFP or GFP. All of the recorded red fluorescence-emitting cells expressed ASIC1a. In whole cell recordings, cells were held at −60 mV at pH 7.4 and then submitted to a drop in external pH for a 5-s time period before return to pH 7.4. In the presence of p11, an ∼2-fold increase in ASIC1a current density was observed compared with the GFP-transfected controls (Fig. 4A). The doubling of ASIC1a current density occurred between pH 6.5 and 5. At pH values more basic than 6.5, ASIC1a was not activated, and pH values more acidic than 5 were not tested, as ASIC1a reached its maximal activation at pH 5.5 (Fig. 4B). The increases in ASIC1a current density were 2.07-fold at pH 6.5 (from 98.98 ± 35 to 204.93 ± 27 pA/pico farads), 2.11-fold at pH 6 (from 358.15 ± 103 to 756.12 ± 170 pA/pico farads), 1.96-fold at pH 5.5 (from 495.46 ± 114 to 972.74 ± 216 pA/pico farads), and 1.94-fold at pH 5 (from 411.04 ± 107 to 797.4 ± 159 pA/pico farads). The mean peak current densities in the absence and presence of p11 and a Hill coefficient of 2.42 and a half-activation pH of 6.26 in the absence of p11 and a Hill coefficient of 2.72 and a half-activation pH of 6.3 in the presence of p11 (Fig. 4B,C). These figures are not significantly different (Fig. 4C), meaning that p11 had no effect on the activation of ASIC1a. We next checked whether p11 affects the desensitization of ASIC1a by measuring the desensitization time constants resulting from the fit of the decaying phase of the currents with single exponentials. At all tested pH values, the desensitization time constants were almost identical with and without p11 (Fig. 4D). Therefore, p11 alters ASIC1a current density (increases the number of channels being delivered to the cell surface) while keeping the intrinsic properties of the channel (activation and desensitization) unchanged.

To assess the specificity of the functional effect of p11 on ASIC1a, we carried out the same experiments with DRG-specific ASIC3. Cells were held at −60 mV, and ASIC3 was activated by an acid challenge (Fig. 5A). Peak ASIC3 current density was not changed by overexpression of p11 (Fig. 5A and B), and p11 did not have any effect on the desensitization kinetics (Fig. 5C). The ASIC3 current is characterized by two distinct phases: a transient current activated by slightly acidic pH followed by a sustained current activated at lower pH. Under our conditions (pH 6), the activation level of the transient current was nearly maximal. Although much less, the sustained current was readily activated and large enough to allow the observation of any change in its amplitude. Again, no statistically significant change was observed in the current density of the sustained phase of ASIC3 in presence of p11 (Fig. 5D).

Taken together, these data show that p11 has no functional effect on ASIC3 and are consistent with the yeast two-hybrid screen, pull-down assay, immunocytochemistry, and biotinylation data. All of the data confirm a functional effect of the p11 interaction with ASIC1a (p11 targets functional channels to the plasma membrane) and show the specificity of the functional interaction between ASIC1a and p11 at the level of the whole channel (i.e. p11 does not functionally interact with the whole ASIC3 channel).

**DISCUSSION**

In this study, we have shown that p11 binds to ASIC1a and is part of the trafficking machinery that targets the channel to the plasma membrane. Yeast two-hybrid assays, together with pull-down experiments and co-immunoprecipitation from mouse DRGs, demonstrated that p11 bound to the N-terminal region that comprises the first 43 amino acids of rASIC1a. Immunocytochemistry combined with biotinylation experiments showed that this interaction was responsible for more channels being delivered to the cell surface. Finally, whole cell patch clamp recordings demonstrated that p11 specifically targeted functional ASIC1a channels to the plasma membrane.

Several studies (most using a yeast two-hybrid system) have already identified ASIC-interacting proteins. In sensory neurons and in the brain, the PDZ domain-containing protein PICK-1 was shown to interact with the C termini of both ASIC1 and ASIC2 (30, 31), and the interaction of ASIC1 with PICK-1 was found to be modulated via a
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![Diagram of ASIC1α current density](Image)

FIGURE 4. p11 Increases ASIC1α current density. A, representative ASIC1α current traces recorded from CHO-K1 cells held at −60 mV and cotransfected with ASIC1α and GFP (left) or ASIC1α and p11-GFP (right). The currents were activated by a drop in pH from 7.4 to 5 for 5 s. Dashed lines, I = 0. B, mean peak ASIC1α current density at −60 mV as a function of extracellular pH in the absence (○; n = 4, 11, 15, 12, and 21 from pH 7 to 5) and presence (●; n = 4, 10, 13, 10, and 20 from pH 7 to 5) of transfected p11. *, p ≤ 0.05. PF. picrofarnaisi. C, relative peak ASIC1α current densities normalized to the peak ASIC1α current density at pH 5 in the absence (○) and presence (●) of transfected p11. The holding potential was −60 mV. D, mean time constants of ASIC1α current desensitization (τ) derived from single exponential fits of the decaying phase of the currents as a function of extracellular pH in the absence (○) and presence (●) of transfected p11.

Here, we have shown that p11 increases ASIC1α expression at the cell surface. p11 (known as annexin II light chain and S100A10), a 95- or 97-amino acid protein in rat and human, respectively, is a member of the S100 family protein. S100 proteins are small non-ubiquitous phospholipid- and Ca2+-binding proteins that exhibit two EF-hand Ca2+-binding sites (39). They have no known enzymatic activities (40) and are involved in many cellular processes such as Ca2+ homeostasis, phospholipid-protein interactions, transcription factor regulation, inflammatory response, cAMP signaling, cytoskeletal dynamics, and cell proliferation and differentiation (39). To be biologically active, the S100 proteins have to be expressed as dimers, and this process is Ca2+-dependent. However, p11 is an exception. Two mutations in the EF-hands make it constitutively active, and Ca2+ is not required for its noncovalent dimerization. Under nonreducing conditions such as those found in the extracellular space, dimers of p11 can be disulfide-linked and form tetramers. Such oxidized forms of S100 proteins have been detected in the extracellular milieu (41). Intracellularly, S100 proteins function through Ca2+-dependent interactions with target proteins. p11 is often found tightly associated by hydrophobic interactions with annexin A2 (also known as annexin II heavy chain, p36, and calpactin I) in the form of a tetrameric complex, (p11)2-(p36)2 (41). Nevertheless, because of its conformation that resembles that of Ca2+-loaded EF-hands, p11 is able to bind the N terminus of annexin A2 with high affinity in the absence of calcium (40). Although abundant intracellularly, in vascular endothelial cells, the annexin A2-p11 complex is also able, via a p11-dependent mechanism, to translocate into the plasma membrane, where it serves as a plasminogen receptor (42).

More generally, annexin A2 expressed in a heterotetrameric form with p11 binds via its C terminus to F-actin and is able to bundle F-actin filaments (43). The tetrameric annexin A2-p11 complex has been shown to be involved in the regulation of membrane organization (i.e. stabilization of lipid rafts), membrane-cytoskeleton linkage, membrane trafficking, and endocytosis (43). The specific distribution of the annexin A2-p11 complex in lipid rafts of dynamic actin-rich structures suggests an important and specialized role for ASIC1α in such structures both at the level of sensory terminals and in brain synapses.

p11 is known to interact with other ion channels. Disruption of the annexin A2-p11 complex was shown to decrease volume-activated chloride currents in vascular endothelial cells (44). p11 promotes the insertion into the plasma membrane of TASK-1 background K+ channels (28) as well as TRPV5 and TRPV6 channels (29) via an interaction with a type I PDZ domain-binding sequence on the C terminus of all three channels; in the case of TASK-1, the binding of p11 causes an endolysosomal reticulum retention signal to be masked (28). p11 also targets Na+,H+–SNS tetrodotoxin-resistant Na+ channels to the plasma membrane of sensory neurons, but this time via an interaction with the Na+,H+–SNS tetrodotoxin-resistant Na+ channels to the plasma membrane of sensory neurons, but this interaction is also not dependent on the Na+,H+–SNS tetrodotoxin-resistant Na+ channel, which, like the ASIC1α N terminus, does not contain a type I PDZ domain-binding site. The binding of p11 to Na+,H+–SNS tetrodotoxin-resistant Na+ channel is also not required for its noncovalent dimerization. Under nonreducing conditions such as those found in the extracellular space, dimers of p11 can be disulfide-linked and form tetramers. Such oxidized forms of S100 proteins have been detected in the extracellular milieu (41). Intracellularly, S100 proteins function through Ca2+-dependent interactions with target proteins. p11 is often found tightly associated by hydrophobic interactions with annexin A2 (also known as annexin II heavy chain, p36, and calpactin I) in the form of a tetrameric complex, (p11)2-(p36)2 (41). Nevertheless, because of its conformation that resembles that of Ca2+-loaded EF-hands, p11 is able to bind the N terminus of annexin A2 with high affinity in the absence of calcium (40). Although abundant intracellularly, in vascular endothelial cells, the annexin A2-p11 complex is also able, via a p11-dependent mechanism, to translocate into the plasma membrane, where it serves as a plasminogen receptor (42).

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sequence 1–43 of ASIC1a does not show any similarity, suggesting that the interaction of p11 with ASIC1a is likely to be different from that with Na$_1$1.8, raising the interesting possibility that more than one channel sequence may interact with the same p11 molecule.

The ASIC1a-p11 interaction is physiologically relevant, as p11 is expressed in nerve tissues expressing ASIC1a, i.e. somatic and visceral sensory neurons (27, 46) and brain (47). Hence, mediators that are released in that tissues and that regulate p11 expression may also modulate the expression of functional ASIC1a channels. Nerve growth factor (27), nitric oxide (48), glucocorticoids (49), and vitamin D (29) are known to up-regulate p11 expression. In response to tissue damage, pro-inflammatory nerve growth factor and anti-inflammatory gluco-corticoids are produced, suggesting that they may both be involved in ASIC regulation in DRG neurons during these pathophysiological conditions. p11 is known to have direct anti-inflammatory effects through the blockade of cytoplasmic phospholipase A$_2$ activity, whereas annexin II subunits also down-regulate prostanoid production by lowering phospholipase activity (50). The role of p11 in inflammation may thus be complex: on the one hand, increasing the gain of sensory neurons in areas of tissue damage by increasing the expression of nociceptive channels and, on the other hand, causing feedback inhibition of eicosanoid production by blockade of phospholipase A$_2$ (50).

Finally, during oxygen/glucose deprivation conditions that occur in ischemia, where ASIC1a-mediated cell damage is a major concern (24, 51), reactive oxygen/nitrogen species (including nitric oxide) are produced. Hence, acting via p11, nitric oxide might be responsible for up-regulating ASIC1a during brain ischemia by analogy with the finding that nitric oxide increases TRPM7 activity during oxygen/glucose deprivation (52). Pharmacologically, the association between p11 and ASIC1a is important in that it may allow indirect targeting of ASIC1a expression via p11. This could lead to significant progress in the search for a treatment of brain ischemia as well as pain and digestive disorders.

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