The Cytoskeletal Protein α-Actinin Regulates Acid-Sensing Ion Channel 1a Through a C-Terminal Interaction

Mikael K. Schnizler1,2, Katrin Schnizler3, Xiang-ming Zha1,2, Duane D. Hall3, John A. Wemmie4,6, Johannes W. Hell3, and Michael J. Welsh1,2,5

Howard Hughes Medical Institute1, and Departments of Internal Medicine2, Pharmacology3, Psychiatry4, and Molecular Physiology and Biophysics5 Roy J. and Lucille A. Carver College of Medicine University of Iowa, Iowa City, Iowa 52242
and
6Department of Veterans Affairs Medical Center, Iowa City, IA, 52242

Running Title: α-Actinin regulates ASIC1a

Address correspondence to: Michael J. Welsh
Howard Hughes Medical Institute
500 EMRB
Roy J. and Lucille A. Carver College of Medicine
University of Iowa
Iowa City, IA 52242
Phone 319-335-7619
FAX 319-335-7623
E-mail michael-welsh@uiowa.edu

ABSTRACT

The acid-sensing ion channel-1a (ASIC1a) is widely expressed in central and peripheral neurons where it generates transient cation currents when extracellular pH falls. ASIC1a confers pH-dependent modulation on postsynaptic dendritic spines and has critical effects in neurological diseases associated with a reduced pH. However, knowledge of the proteins that interact with ASIC1a and influence its function is limited. Here, we show that α-actinin, which links membrane proteins to the actin cytoskeleton, associates with ASIC1a in brain and in cultured cells. The interaction depended on an α-actinin-binding site in the ASIC1a C-terminus that was specific for ASIC1a vs.

other ASICs and for α-actinin-1 and -4. Co-expressing α-actinin-4 altered ASIC1a current density, pH-sensitivity, desensitization rate, and recovery from desensitization. Moreover, reducing α-actinin expression altered acid-activated currents in hippocampal neurons. These findings suggest that α-actinins may link ASIC1a to a macromolecular complex in the postsynaptic membrane where it regulates ASIC1a activity.

INTRODUCTION

Acid-sensing ion channels (ASICs) are H⁺-gated members of the DEG/ENaC family (1-3). Members of this family contain cytosolic N- and C-termini, two transmembrane domains, and a large cysteine-rich extracellular domain. ASIC subunits combine as homo- or hetero-trimers to
form cation channels that are widely expressed in the central and peripheral nervous systems (1-4). In mammals, four genes encode ASICs, and two subunits, ASIC1 and ASIC2, have two splice forms, -a and -b. Central nervous system (CNS) neurons express ASIC1a, ASIC2a, and ASIC2b (5-7). Homomeric ASIC1a channels are activated when extracellular pH drops below 7.2, and half-maximal activation occurs at pH 6.5-6.8 (8-10). These channels desensitize in the continued presence of a low extracellular pH, and they can conduct Ca\(^{2+}\) (9,11-13). ASIC1a is required for acid-evoked currents in CNS neurons; disrupting the gene encoding ASIC1a eliminates H\(^+\)-gated currents unless extracellular pH is reduced below pH 5.0 (5,7).

Previous studies found ASIC1a enriched in synaptosomal membrane fractions and present in dendritic spines, the site of excitatory synapses (5,14,15). Consistent with this localization, ASIC1a null mice manifested deficits in hippocampal long-term potentiation, learning, and memory, which suggested that ASIC1a is required for normal synaptic plasticity (5,16). ASICs might be activated during neurotransmission when synaptic vesicles empty their acidic contents into the synaptic cleft or when neuronal activity lowers extracellular pH (17-19). Ion channels, including those at the synapse often interact with multiple proteins in a macromolecular complex that incorporates regulators of their function (20,21). For ASIC1a, only a few interacting proteins have been identified. Earlier work indicated that ASIC1a interacts with another postsynaptic scaffolding protein, PICK1 (15,22,23). ASIC1a also has been reported to interact with annexin II light chain p11 through its cytosolic N-terminus to increase cell surface expression (24) and with Ca\(^{2+}\)/calmodulin dependent protein kinase II to phosphorylate the channel (25). However, whether ASIC1a interacts with additional proteins and with the cytoskeleton remain unknown. Moreover, it is not known whether such interactions alter ASIC1a function.

In analyzing the ASIC1a amino acid sequence, we identified cytosolic residues that might bind \(\alpha\)-actinin. \(\alpha\)-Actinins cluster membrane proteins and signaling molecules into macromolecular complexes and link membrane proteins to the actin-cytoskeleton (reviewed in (26)). Four genes encode \(\alpha\)-actinin-1, -2, -3, and -4 isoforms. \(\alpha\)-Actinins contain an N-terminal head domain that binds F-actin, a C-terminal region containing two EF-hand motifs, and a central rod domain containing four spectrin-like motifs (26-28). The C-terminal portion of the rod segment appears to be crucial for binding to membrane proteins. The \(\alpha\)-actinins assemble into antiparallel homo-dimers through interactions in their rod domain. \(\alpha\)-Actinins-1, -2, and -4 are enriched in dendritic spines, concentrating at the postsynaptic membrane (29-35). In the postsynaptic membrane of excitatory synapses, \(\alpha\)-actinin connects the NMDA receptor to the actin cytoskeleton, and this interaction is key for Ca\(^{2+}\)-dependent inhibition of NMDA receptors (36-38). \(\alpha\)-Actinins can also regulate the membrane trafficking and function of several cation channels, including L-type Ca\(^{2+}\) channels, K\(^+\) channels, and TRP channels (39-41).

In order to better understand the function of ASIC1a channels in macromolecular complexes, we asked if ASIC1a associates with \(\alpha\)-actinins. We were interested in the \(\alpha\)-actinins because both they and ASIC1a are present in dendritic spines, ASIC1a contains a potential \(\alpha\)-actinin binding sequence, and the related epithelial Na\(^+\) channel (ENaC) interacts with the cytoskeleton (42,43). Therefore, we hypothesized that \(\alpha\)-actinin interacts structurally and functionally with ASIC1a.
EXPERIMENTAL PROCEDURES

Expression constructs.
Mouse ASIC1a was cloned into pMT3 (44) for heterologous expression. Human α-actinin-1 was a gift of C. Otey, University of North Carolina and α-actinin-2, -3, and -4 were gifts of M. Sheng, Massachusetts Institute of Technology. Full length human α-actinin-1 through -4 were cloned into pEGFP vectors (Clontech Laboratories) to generate EGFP-α-actinin fusion proteins; previous studies showed that the localization and function of α-actinins were unaffected by GFP-tags (45-47). A dominant-negative N-terminally truncated (missing amino acid 3-249) human α-actinin-1 (rod-actinin) (48) was a gift of A. Huttenlocher, University of Wisconsin. Rod-actinin contains the central α-actinin spectrin domain including the C-terminus, but lacks the N-terminal head-domain. Dimers containing rod-actinin can bind other proteins, but the absence of the head domain interrupts crosslinking to the filamentous actin cytoskeleton (48,49). Rod-actinin would be expected to interfere with all α-actinins, and consistent with that conclusion, it disrupted the effect of α-actinins in HEK cells, which express both α-actinin-1 and -4 (JWH, unpublished observation). CHO cells also express α-actinin-1 and α-actinin-4 (50). HA-tagged ASIC1a was made by adding the influenza hemagglutinin epitope (YPYDVPDYAGV) to the N-terminus of ASIC1a.

Cell culture and transfection.
For patch-clamp studies Chinese hamster ovary (CHO) cells were transfected with 1-6 µg DNA using the TransFast Lipid™ Reagent (Promega Madison, WI) and cultured on glass cover slips in 35-mm Petri dishes. To identify transfected cells using epifluorescence, we used enhanced green fluorescent protein (EGFP) at an ASIC1a:EGFP ratio of 6:1. Channel and α-actinin constructs were transfected at 1:1 ratio. Empty vector DNA was used to maintain a constant final DNA concentration for all transfections. Experiments were performed at room temperature 2-3 days after transfection.

Hippocampal neuronal culture and transfection. Rat E18 hippocampal neurons (Brain Bits, Springfield, IL) were transfected after 7–8 days in culture using Ca²⁺-phosphate transfection. Conditioned medium was collected and replaced by freshly prepared neurobasal medium with B-27 supplement (Gibco, Invitrogen) 30 min prior to transfection. A total of 5 µg vector DNA was added in a transfection mix consisting of 50 ml water, 5 ml CaCl₂ (2.5 M) and 60 ml of 2xPBS per dish. After 3 h incubation at 37 °C and following 8 min HBSS washing step, conditioned media was reapplied to cultures. At day 10, neurons were used for whole-cell, patch-clamp experiments.

Immunoprecipitation and immunoblotting.
Whole mouse brains from adult animals were homogenized in ice-cold buffer (5 ml/brain) containing 300 mM sucrose, 10 mM Tris (pH 7.5), 20 mM NaCl plus protease inhibitors (Complete Mini, EDTA-free, Roche) using a glass-teflon homogenizer. Samples were spun for 2 min in a tabletop centrifuge at 2000 x g to remove large debris. Supernatants were centrifuged for 30 min at 135,000 x g in a Beckman TLA100.3 rotor at 4 °C. The membrane pellet was washed once on ice and then solubilized in 6 ml of 50 mM Tris (pH 7.5), 150 mM NaCl, protease inhibitors (Complete Mini) with 1 % Triton X-100. For immunoprecipitation, samples were centrifuged (10 min, 700 x g) to remove particulate debris and pre-cleared by addition of protein G agarose (Roche, 50 ml, 15 mg/ml). Either undiluted affinity-purified ASIC1 antibody (raised in rabbits against the 22 amino acid peptide from the C terminus of ASIC1 and purified as described previously (16) or a mouse monoclonal α-actinin antibody BM-75.2 that...
binds to all four actinin isoforms (1 ml, Sigma-Aldrich) were added to 750 ml of protein extract and incubated for 3 h while rotating at 4°C. For precipitation, Protein G agarose beads (Roche, 50 ml 15 mg/ml) were added and samples were rotated over night at 4 °C. After centrifugation, three wash steps were performed to clear samples (buffer 1: 50 mM Tris (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 0.5% Na-deoxycholate, protease inhibitors (Complete Mini); buffer 2: 50 mM Tris (pH 7.5), 500 mM NaCl, 0.1% Nonidet P-40, 0.05% Na-deoxycholate; buffer 3: 10 mM Tris (pH 7.5), 0.1% Nonidet P-40, 0.05% Na-deoxycholate). Bound proteins were extracted at 95 °C in sodium dodecyl sulfate (SDS)-sample buffer (0.125 mM Tris (pH 7.5), 3.4% SDS, 17% glycerol, 67 mM dithiothreitol, 0.008% bromphenol blue) for 10 min and were resolved by SDS-PAGE and immunoblotted with the indicated antibodies.

Co-immunoprecipitation from COS-7 cells was performed 48 h after electroporation (10⁶ cells, 10 or 15 µg of DNA at 1:1 ASIC1a:α-actinin ratio per sample). Electroporation was performed with 400 µl of prechilled cell suspension (in [mM]), 120 KCl, 25 HEPES, 0.15 NaCl, 10 KPO₄, 2 EGTA, 5 MgCl₂, with 2 mM ATP and 5 mM glutathione using a single 25 sec pulse of 0.320 V and 975 µF. Cells in 100 mm dishes were washed twice with ice-cold PBS and harvested in lysis buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, and protease inhibitors (Complete Mini). Supernatants from cell extracts were precleared with Protein-A sepharose (Pierce) gently agitated for 1 h at 4°C. Either ASIC1 antibody (16) or rabbit polyclonal anti-GFP antibody (Clontech, Living Colors®Full-length A.v. polyclonal Antibody) were added to 250-500 µl of protein extract after 1 h of rotation at 4 °C. Protein A sepharose was added followed by incubation overnight with rotation at 4 °C. Immunoprecipititates absorbed to protein A sepharose were washed three times as described above. The immunoprecipitated proteins were resuspended in SDS-sample buffer and electrophoresed. For immunoblotting primary antibodies were detected with HRP-conjugated goat-anti rabbit or anti-mouse IgG from ECL (GE Healthcare, UK). SuperSignal West Pico Chemiluminescent Substrate (Pierce) was used for detection.

CHO cells were transfected with HA-ASIC1α:α-actinin:GFP at 1:1:1 ratio. For control, α-actinin was replaced with GFP so the ratio was 1:2 for HA-ASIC1α:GFP.

Biotinylation of CHO cells was performed two days after lipofectamine-mediated transfection. Cells were washed 3x with ice-cold PBS++, and 3 ml of 0.5 mg/ml NHS-biotin in PBS++ was added to each 10 cm dish, followed by incubation at 4°C for 30 min with gentle rocking. Cells were washed once with PBS++, and 0.1 M glycine in PBS++ was added to quench the reaction, followed by 2 washes with PBS++. Cells were lysed in PBS with 1% NP40, 0.5% deoxycholate, 0.5% SDS and freshly added proteinase inhibitors (Roche). Cell lysate was sonicated briefly and cleared by centrifugation. Protein concentration was quantified using a modified Bradford assay kit (Bio-Rad). For Neutravidin pulldown, 60 µl of a 50% slurry of Neutravidin beads were added to 200 µl of cell lysate and incubated at 4°C overnight with gentle rotation. Beads were washed 3x with wash buffer (Tris 50 mM, pH 7.4, 1% Triton X-100). Beads were then boiled in 80 µl of SDS sample buffer with or without reducing agent.

RT-PCR.

Rat hippocampal neurons and rat muscle tissue were lysed in cell lysis buffer and first strand cDNA was synthesized using the Cells-to-cDNA II Kit (Ambion). For PCR, the primer pairs for detection of α-actinin-1 were 5’-gatgcagacaaggagcgct-3’ (bp:1887-1906) and
5’-gggacccaacgtgcggag-3’ (bp: 2495-2513); for α-actinin-2 they were 5’-agaatgaggtggagaagttga-3’ (bp: 1787-1807) and 5’-ttggagagctgcgttccttg-3’ (bp: 2467-2486); for α-actinin-3 they were 5’-ctgcagctggttgctcg-3’ (bp: 1607-1625) and 5’-tgctgctgcatgtacc-3’ (bp: 2196-2215); and for α-actinin-4 they were 5’-agcaatcacatacagtcg-3’ (bp: 1906-1927) and 5’-ccacactcagctgcgttg-3’ (bp: 2542-2561). RT-PCR products were sequenced to confirm identity.

**RNA interference.**

We generated small interfering RNAs (siRNAs) against each of the four rat α-actinin isoforms using OligoEngine™ to locate 19 nucleotides within exons of the target and immediately downstream of AA dinucleotides and to exclude identity to other sequences in the NCBI database. Sequences with potential Pol III termination sites and mRNA splice sites were avoided.

The 19-nucleotide sense sequence and the inverted anti-sense sequence were connected by a nine nucleotide spacer to allow stem-loop formation. At the 3’ end, a pentathymidine motif provided a Pol III termination site. siRNAs were cloned into pSilencer™ vector (Ambion) for expression of the respective sequences under the U6 promoter. DsRed and its CMV promoter from pDsRed2-N1 were cloned into the Kpn site upstream of the U6 promoter within the pSilencer™ vector; this vector was used for identification of transfected neurons. To test for efficacy and specificity of siRNA, CHO cells were co-transfected with either one of the siRNAs and EGFP-tagged α-actinin at a 10:1 cDNA ratio (total 11 mg DNA) using Lipofectamine 2000 in OptiMEMI media according to the manufacturer’s recommendation (Invitrogen). RNA1 targeted at 476-494 from the start sequence of rat α-actinin-1 (the sense chain was 5’-gatctcaaacaagtgtgaggttgaggatcttttt-3’. RNA2 targeted at 224-242 from the start sequence of rat α-actinin-2; the sense chain was 5’-tgctgtgcatgtcagctacagctgcatctcaggtc-3’. RNA3 targeted at 455-473 from the start sequence of rat α-actinin-3; the sense chain was 5’-aagagaagctgtggagaagctgcatgtcagctgcatctcagctgcatctcaggtc-3’. RNA4 targeted at 493-511 from the start sequence of rat α-actinin-4; the sense chain was 5’-gactatcaggagatgctgcagctgtggagatcttttt-3’.

**Electrophysiology.**

Whole-cell, patch-clamp recordings were performed on CHO cells on 10 mm glass coverslips continuously superfused with a bath solution containing (in mM): 128 NaCl, 5.4 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, 10 MES, 5.55 glucose, adjusted to pH 7.4 with TMAOH. The standard pipette solution contained (in mM): 10 NaCl, 121 KCl, 5 EGTA, 2 MgCl2, 2 Na2-ATP, 10 HEPES adjusted to pH 7.25 with TMAOH. For whole-cell, patch-clamp experiments with hippocampal neurons the bath solution contained (in mM): 100 NaCl, 5.4 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, 10 MES, 10 glucose, adjusted to pH 7.4 with TMAOH. The pipette solution contained (in mM): 10 NaCl, 70 K-glucorate, 10 KCl, 10 EGTA, 1 MgCl2, 3 Na2-ATP, 25 HEPES adjusted to pH 7.25 with TMAOH. Bath solutions with different pH values were adjusted with TMAOH and applied with a rapid solution exchanger (RSC-200 & EVH-9, Biologic Science Instruments). Recording pipettes were pulled from capillary glass with a micropipette puller (Sutter instruments) and polished (MF830, Narishige, Japan). Pipette resistances ranged from 2-5.5 MΩ.

Recordings were made at room temperature using an AXOPATCH 200B amplifier with pCLAMPex 8.1 software (Axon Instruments).
These data suggest that ASIC1a resides in close proximity with at least two of the actinins, α-actinin-1 and α-actinin-4.

To learn whether the potential α-actinin-binding motif in the C-terminus of ASIC1a was involved in the interaction between ASIC1a and α-actinin, we expressed α-actinin-4 with an ASIC1a variant that contained mutations in the potential α-actinin binding motif (ASIC1a\textsubscript{mut} residues 484\textasciitilde490 mutated to 484\textasciitilde490). Mutating the C-terminal motif prevented ASIC1a from co-precipitating α-actinin-4 (Fig. 3). Likewise, α-actinin-4 failed to co-precipitate ASIC1a\textsubscript{mut}. These results suggest a direct association between α-actinin-4 and ASIC1a that depends on the α-actinin-binding motif. For most of the remainder of the studies, we focused on α-actinin-4.

α-Actinin did not affect cell surface expression of ASIC1a.
To determine whether α-actinin might regulate surface expression of ASIC1a, we biotinylated cell surface proteins in CHO cells transfected with ASIC1a. Co-expressing ASIC1a with α-actinin-4, α-actinin-1 or rod-actinin did not alter the fraction of ASIC1a on the cell surface (Fig. 4A, 4B).

α-Actinin influences ASIC1a current density.
To learn whether α-actinin also regulates the function of ASIC1a, we co-expressed them and measured currents. α-Actinin-4 reduced ASIC1a current density, whereas α-actinin-1 had no effect (Fig. 5A, 5B). These results suggest that even though both α-actinins interact with ASIC1a, they have selective functional effects. As an additional test of the effect of α-actinin, we co-expressed ASIC1a with rod-actinin, a dominant-negative construct (48,49). We found that the dominant-negative rod-actinin fragment had the opposite effect of α-actinin-4 and increased current density (Fig. 5A, 5B).
Finding that rod-actinin increased current amplitude suggested that it disrupted an interaction between ASIC1a and an endogenous α-actinin. Such an interaction predicts that ASIC1a<sub>mut</sub>, which did not interact with α-actinin, would have a greater current density than ASIC1a. Consistent with this idea, ASIC1a<sub>mut</sub> generated more current than ASIC1a (Fig. 5A, 5B). In addition, α-actinin-1 and α-actinin-4 failed to alter current produced by ASIC1a<sub>mut</sub>. These data also suggest that the effect of α-actinin-4 on current results from a direct interaction with ASIC1a.

**α-Actinin alters the properties of ASIC1a currents.**
The findings that α-actinin-4 did not change the amount of ASIC1a on the cell surface, whereas it decreased current density suggested that α-actinin-4 must have also altered the properties of the current generated by ASIC1a or increased the proportion of silent channels. To test this possibility, we examined several characteristics of ASIC1a current, including the pH-sensitivity, the rate of desensitization, and the time course of recovery from desensitization.

Co-expressing α-actinin-4 increased the pH-sensitivity of ASIC1a (Fig. 6A, Table 1). Conversely, the dominant-negative rod-actinin had the opposite effect, reducing pH-sensitivity. Consistent with these data, when we disrupted the ASIC1a α-actinin-binding site (ASIC1a<sub>mut</sub>), pH-sensitivity fell, and α-actinin-4 and rod-actinin failed to alter the pH-sensitivity of this variant (Fig. 6B, Table 1). These results indicate that the interaction with α-actinin influenced the sensitivity of ASIC1a to extracellular protons.

Following acid-evoked activation, ASIC1a currents desensitize in the continued presence of acid (Fig. 5A). We found that neither α-actinin-1 nor -4 coexpression altered the time constant of ASIC1a current desensitization (τ<sub>des</sub>) (Fig. 7). However, disrupting the α-actinin-binding site in ASIC1a<sub>mut</sub> or co-expressing rod-actinin with ASIC1a increased τ<sub>des</sub>. Moreover, disrupting the α-actinin-binding motif prevented the effect of rod-actinin. These data suggest that α-actinin can alter the desensitization rate of ASIC1a channels, an effect that is mediated in part by interactions between ASIC1a and ubiquitously expressed α-actinins.

ASIC1a currents also show a characteristic time-dependent recovery from desensitization in acidic solution (8,10). We activated and desensitized ASIC1a currents with a 60 sec exposure to pH 6.0 solution (Fig. 8A). Then, after varying time intervals at pH 7.4, we re-exposed the channels to pH 6.0. Co-expressing α-actinin-4 accelerated recovery from desensitization (Fig. 8B). Conversely, co-expressing a dominant-negative rod-actinin with ASIC1a or disrupting the α-actinin-binding motif with ASIC1a<sub>mut</sub> slowed the rate of recovery.

These results indicate that an association with α-actinin-4 altered several characteristics of ASIC1a channel function. They also implicate the α-actinin-binding motif in the ASIC1a C-terminus. The effects of the dominant-negative rod domain and the ASIC1a<sub>mut</sub> variant suggest that ASIC1a interacts with α-actinins that are endogenous in the heterologous cells we used to express ASIC1a. Therefore, in the studies described below, we tested for an effect of endogenous α-actinins.

**ASIC1a and α-actinin associate in brain.**
To test the hypothesis that ASIC1a and α-actinins associate in neurons, we used immunoprecipitation from mouse brain. We found that ASIC1a co-precipitated α-actinin, and α-actinin co-precipitated ASIC1a (Fig. 9A). α-actinin failed to co-immunoprecipitate
ASIC1a from ASIC1a-/ mouse brain (Fig. 9B). These results suggest that ASIC1a and α-actinin reside in close proximity in brain.

**α-Actinin regulates hippocampal acid-activated currents.**

In the brain, ASIC1a has been implicated in learning and memory and an association of ASIC1a with the cytoskeletal linker protein α-actinin could be one mechanism underlying synaptic plasticity. Our data suggested that in heterologous cells endogenous α-actinins regulate ASIC currents; therefore we asked if this was the case in neurons. We used RT-PCR to learn which **α-actinin** genes are expressed in the hippocampus and found **α-actinin-1, -2 and -4**, but not **α-actinin-3** (Fig. 10A). To reduce their expression, we constructed vectors encoding siRNAs directed against each **α-actinin** isoform. The efficacy and specificity of the RNA interference were shown by western blotting (Fig. 10B). With this information, for subsequent studies we reduced α-actinin expression by co-transfecting primary cultures of hippocampal neurons with a mix of the siRNAs against **α-actinin-1, -2 and -4** (RNA, to **α-actinin-1,2,4**). As controls, we used either siRNA directed against **α-actinin-3** or dsRed.

Acid-evoked currents in hippocampal neurons are produced by a mixture of ASIC1a, -2a and -2b subunits (7,53,54). RNA, against **α-actinin-1,2,4** increased endogenous ASIC current density in hippocampal neurons (Fig. 10C). In addition, it reduced the pH-sensitivity of hippocampal acid-activated currents (Fig. 10D). Although the effects were small, it also slowed the rate of recovery from desensitization within the first 2 sec (Fig. 10E). These results are consistent with our findings in a heterologous system expressing ASIC1a alone. In contrast to our results with ASIC1a homomultimers studied in CHO cells, τdes of hippocampal ASIC currents were not affected by reducing endogenous α-actinins (Fig. 10F).

**DISCUSSION**

Previous studies localized α-actinin to dendritic spines and the postsynaptic density (29-35). Likewise, ASIC1a localized to dendritic spines and was enriched in the postsynaptic membrane (5,14,15). Those earlier studies positioned α-actinin-4 and ASIC1a where they could interact. In this study, we now show physical and functional links between these two proteins both in heterologous cells and in neurons. The data also indicate that the ASIC1a:α-actinin interaction occurred through a motif in the ASIC1a C-terminus. This interaction appears to be specific, as the α-actinin-binding motif is not conserved in ASIC2 or -3. In addition, ASIC1a associated with α-actinin-1 and -4, but not with α-actinin-2 or -3. These results suggest specificity in the interaction between these two families of proteins.

The association with α-actinin likely incorporates ASIC1a into a postsynaptic macromolecular signaling complex. α-actinins bind actin and may thereby link ASIC1a to the predominant cytoskeletal element of dendritic spines (55,56). The C-termini of α-actinin also contain PDZ-binding motifs that attach to several scaffolding proteins, including densin-180 (21,33,57). Thus, α-actinin may tie ASIC1a to multiple other proteins to form a signaling complex in the postsynaptic membrane (21). A potential example (25) is the functional coupling between ASIC1a and NMDA receptors; NMDA receptors are also regulated by α-actinin (38,58). Gao et al. (25) suggested that during acidosis, Ca2+/calmodulin-dependent protein kinase II is recruited to NMDA receptors where it phosphorylates ASIC1a, thereby increasing its pH-sensitivity. Interestingly, ASIC1a (5), α-actinin (37), NMDA receptors (58), actin (59), and many of
their associated components contribute to synaptic plasticity.

The mechanism by which α-actinin-4 reduced current density appears to be a direct effect on channel function. This conclusion is supported by the finding that α-actinin-4 altered multiple characteristics of the current without changing the amount of ASIC1a on the cell surface. In CHO cells, overexpressing α-actinin-4 increased H⁺-evoked current density and increased pH-sensitivity, whereas we saw the opposite effect when we expressed the dominant-negative rod-actinin or mutated the α-actinin interaction motif in ASIC1a. Likewise, in neurons, knockdown of α-actinin increased current density and reduced pH-sensitivity. However, there were some differences between H⁺-gated currents in CHO cells expressing ASIC1a and H⁺-gated currents in neurons. In CHO cells, α-actinin-4 accelerated and rod-actinin and ASIC1a mut slowed recovery from desensitization, whereas α-actinin siRNA had minor effects on recovery from desensitization in neurons. Moreover, the interaction with α-actinin altered the desensitization rate of ASIC1a currents in CHO cells, but α-actinin siRNA had no effect on H⁺-activated current in neurons. We suspect these differences result from the fact that in CHO cells we studied ASIC1a homomeric channels, whereas in neurons much of the ASIC1a exists in heteromultimers (7). The conclusion that α-actinin-4 increased pH-sensitivity in both cases is interesting, because pH-sensitivity is a property usually associated with ASIC’s extracellular domain (4,60-62). Therefore, we speculate that the α-actinin binding motif in the ASIC1a intracellular C-terminus somehow links ASIC1a to structures that control gating and pH-sensitivity.

α-Actinin is not the only protein that interacts with ASIC1a; PICK1 (protein interacting with C kinase), stomatin, and annexin II light chain p11 also bind the channel. In PICK1, a PDZ domain interacts with the ASIC1a C-terminus, and the two proteins resided in dendrites, probably in postsynaptic membranes (15,22). Stomatin associated with ASIC1a in heterologous systems, although the site of interaction remains unknown (63). Neither PICK1 nor stomatin altered the amplitude or properties of ASIC1a currents. The annexin II light chain p11 associated with an undefined site in the ASIC1a N-terminus (24). In heterologous cells, it increased cell surface expression of ASIC1a and increased ASIC1a current amplitude without altering the characteristics of the current. Thus, of the proteins known to associate with ASIC1a, only α-actinin affected its channel function.

The connections between α-actinin and ASIC1a, and their previously established distribution in the postsynaptic membrane, position these proteins at a key site for influencing neuronal activity. Together, these observations suggest that α-actinins may have an important impact on ASIC1a function at this location.

ACKNOWLEDGEMENTS
We thank M. Sheng, C. Otey and A. Huttenlocher for providing α-actinin cDNAs. We thank the In Vitro Models and Cell Culture Core [supported in part by NHLBI (HL61234 and HL515670), the Cystic Fibrosis Foundation (R458-CR02 and ENGLH9850), and NIDDK (DK54759)]. This work was supported in part by Deutsche Forschungsgemeinschaft DFG Forschungsstipendium (Schn 740/2-1) to MKS, AHA #0535235N to DDH, and NIH grant AG017502 to JWH. MJW is an Investigator of the HHMI.
REFERENCES

1. Waldmann, R., Champigny, G., Lingueglia, E., De Weille, J. R., Heurteaux, C., and Lazdunski, M. (1999) *Ann. N. Y. Acad. Sci.* **868**, 67-76
2. Kellenberger, S., and Schild, L. (2002) *Physiol. Rev.* **82**(3), 735-767
3. Wemmie, J. A., Price, M. P., and Welsh, M. J. (2006) *Trends Neurosci.* **29**(10), 578-586
4. Jasti, J., Furukawa, H., Gonzales, E. B., and Gouaux, E. (2007) *Nature* **449**(7160), 316-323
5. Wemmie, J. A., Chen, J., Askwith, C. C., Hruska-Hageman, A. M., Price, M. P., Nolan, B. C., Yoder, P. G., Lamani, E., Hoshi, T., Freeman, J. H. J., and Welsh, M. J. (2002) *Neuron* **34**, 463-477
6. Baron, A., Waldmann, R., and Lazdunski, M. (2002) *J. Physiol.* **539**(Pt 2), 485-494
7. Askwith, C. C., Wemmie, J. A., Price, M. P., Rokhlina, T., and Welsh, M. J. (2004) *J. Biol. Chem.* **279**(18), 18296-18305
8. Benson, C. J., Xie, J., Wemmie, J. A., Price, M. P., Henss, J. M., Welsh, M. J., and Snyder, P. M. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**(4), 2338-2343
9. Xiong, Z. G., Zhu, X. M., Chu, X. P., Minami, M., Hey, J., Wei, W. L., MacDonald, J. F., Wemmie, J. A., Price, M. P., Welsh, M. J., and Simon, R. P. (2004) *Cell* **118**(6), 687-698
10. Babini, E., Paukert, M., Geisler, H. S., and Gründer, S. (2002) *J. Biol. Chem.* **277**(44), 41597-41603
11. Bassler, E. L., Ngo-Anh, T. J., Geisler, H. S., Ruppersberg, J. P., and Grunder, S. (2001) *J. Biol. Chem.* **276**(36), 33782-33387
12. Yermolaieva, O., Leonard, A. S., Schnizler, M. K., Abboud, F. M., and Welsh, M. J. (2004) *Proc. Natl. Acad. Sci. U. S. A.* **101**(17), 6752-6757
13. Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C., and Lazdunski, M. (1997) *Nature* **386**, 173-177
14. Zha, X.-M., Wemmie, J. A., and Welsh, M. J. (2006) *Proc. Natl. Acad. Sci. USA* **103**(44), 16556-16561
15. Hruska-Hageman, A. M., Wemmie, J. A., Price, M. P., and Welsh, M. J. (2002) *Biochem. J.* **361**(Pt 3), 443-450
16. Wemmie, J. A., Askwith, C. C., Lamani, E., Cassell, M. D., Freeman, J. H. J., and Welsh, M. J. (2003) *J. Neurosci.* **23**(13), 5496-5502
17. Miesenbock, G., De Angelis, D. A., and Rothman, J. E. (1998) *Nature* **394**, 192-195
18. Krishtal, O. A., Osipchuk, Y. V., Shelest, T. N., and Smirnoff, S. V. (1987) *Brain Res.* **436**(2), 352-356
19. Chesler, M., and Kaila, K. (1992) *Trends Neurosci.* **15**(10), 396-402
20. Levitan, I. B. (2006) *Nat. Neurosci.* **9**(3), 305-310
21. Kim, E., and Sheng, M. (2004) *Nat Rev Neurosci* **5**(10), 771-781
22. Duggan, A., Garcia-Anoveros, J., and Corey, D. P. (2002) *J. Biol. Chem.* **277**(7), 5203-5208
Leonard, A. S., Yermolaieva, O., Hruska-Hageman, A., Askwith, C. C., Price, M. P., Wemmie, J. A., and Welsh, M. J. (2003) Proc. Natl. Acad. Sci. U. S. A. 100(4), 2029-2034

Donier, E., Rugiero, F., Okuse, K., and Wood, J. N. (2005) J. Biol. Chem. 280(46), 38666-38672

Gao, J., Duan, B., Wang, D. G., Deng, X. H., Zhang, G. Y., Xu, L., and Xu, T. L. (2005) Neuron 48(4), 635-646

Otey, C. A., and Carpen, O. (2004) Cell Motil. Cytoskeleton 58(2), 104-111

Flood, G., Kahana, E., Gilmore, A. P., Rowe, A. J., Gratzer, W. B., and Critchley, D. R. (1995) J Mol Biol 252(2), 227-234

Djinovic-Carugo, K., Gautel, M., Ylannen, J., and Young, P. (2002) FEBS Lett 513(1), 119-123

Wyszynski, M., Kharazia, V., Shanghvi, R., Rao, A., Beggs, A. H., Craig, A. M., Weinberg, R., and Sheng, M. (1998) J Neurosci 18(4), 1383-1392

Rao, A., Kim, E., Sheng, M., and Craig, A. M. (1998) J Neurosci 18(4), 1217-1229

Allison, D. W., Gelfand, V. I., Spector, I., and Craig, A. M. (1998) J Neurosci 18(7), 2423-2436

Asanuma, K., Kim, K., Oh, J., Giardino, L., Chabanis, S., Faul, C., Reiser, J., and Mundel, P. (2005) J Clin Invest 115(5), 1188-1198

Walikonis, R. S., Ogumi, A., Khorosheva, E. M., Jeng, C. J., Asuncion, F. J., and Kennedy, M. B. (2001) J Neurosci 21(2), 423-433

Nuriya, M., Oh, S., and Huganir, R. L. (2005) J. Neurochem. 95(2), 544-552

Nakagawa, T., Engler, J. A., and Sheng, M. (2004) Neuropeharmacology 47(5), 734-745

Rosenmund, C., and Westbrook, G. L. (1993) Neuron 10(5), 805-814

Wyszynski, M., Lin, J., Rao, A., Nigh, E., Beggs, A. H., Craig, A. M., and Sheng, M. (1997) Nature 385(6615), 439-442

Krupp, J. J., Vissel, B., Thomas, C. G., Heinemann, S. F., and Westbrook, G. L. (1999) J Neurosci 19(4), 1165-1178

Sadeghi, A., Doyle, A. D., and Johnson, B. D. (2002) Am J Physiol Cell Physiol 282(6), C1502-1511

Maruoka, N. D., Steele, D. F., Au, B. P., Dan, P., Zhang, X., Moore, E. D., and Fedida, D. (2000) FEBS Lett 473(2), 188-194

Li, Q., Dai, X. Q., Shen, P. Y., Wu, Y., Long, W., Chen, C. X., Hussain, Z., Wang, S., and Chen, X. Z. (2007) J. Neurochem. 103(6), 2391-2400

Mazzochi, C., Benos, D. J., and Smith, P. R. (2006) Am. J. Physiol. Renal Physiol 291(6), F1113-1122

Zuckerman, J. B., Chen, X., Jacobs, J. D., Hu, B., Kleyman, T. R., and Smith, P. R. (1999) J. Biol. Chem. 274(33), 23286-23295

Swick, A. G., Janicot, M., Cheneval-Kastelic, T., McLenithan, J. C., and Lane, M. D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1812-1816

Fraley, T. S., Pereira, C. B., Tran, T. C., Singleton, C., and Greenwood, J. A. (2005) J Biol Chem 280(15), 15479-15482

Zhang, W., and Gunst, S. J. (2006) J Physiol 572(Pt 3), 659-676
47. Rajfur, Z., Roy, P., Otey, C., Romer, L., and Jacobson, K. (2002) Nat Cell Biol 4(4), 286-293
48. Bhatt, A., Kaverina, I., Otey, C., and Huttenlocher, A. (2002) J Cell Sci 115(Pt 17), 3415-3425
49. Wang, J., Shaner, N., Mittal, B., Zhou, Q., Chen, J., Sanger, J. M., and Sanger, J. W. (2005) Cell Motil Cytoskeleton 61(1), 34-48
50. Celli, L., Ryckewaert, J. J., Delachanal, E., and Duperray, A. (2006) J. Immunol. 177(6), 4113-4121
51. Leonard, A. S., Bayer, K. U., Merrill, M. A., Lim, I. A., Shea, M. A., Schulman, H., and Hell, J. W. (2002) J. Biol. Chem. 277(50), 48441-48448
52. Kim, M., Jiang, L. H., Wilson, H. L., North, R. A., and Surprenant, A. (2001) EMBO J. 20(22), 6347-6358
53. Bassilana, F., Champigny, G., Waldmann, R., de Weille, J. R., Heurteaux, C., and Lazdunski, M. (1997) J. Biol. Chem. 272(46), 28819-28822
54. Lingueglia, E., de Weille, J. R., Bassilana, F., Heurteaux, C., Sakai, H., Waldmann, R., and Lazdunski, M. (1997) J. Biol. Chem. 272(47), 29778-29783
55. Dillon, C., and Goda, Y. (2005) Annu Rev Neurosci 28, 25-55
56. Tada, T., and Sheng, M. (2006) Curr Opin Neurobiol 16(1), 95-101
57. Xia, H., Winokur, S. T., Kuo, W. L., Altherr, M. R., and Bredt, D. S. (1997) J Cell Biol 139(2), 507-515
58. Zhang, S., Ehlers, M. D., Bernhardt, J. P., Su, C. T., and Huganir, R. L. (1998) Neuron 21(2), 443-453
59. Cingolani, L. A., and Goda, Y. (2008) Nat. Rev. Neurosci. 9(5), 344-356
60. Paukert, M., Sidi, S., Russell, C., Siba, M., Wilson, S. W., Nicolson, T., and Grunder, S. (2004) J. Biol. Chem. 279(18), 18783-18791
61. Paukert, M., Chen, X., Polleichtner, G., Schindelin, H., and Grunder, S. (2008) J Biol Chem 283(1), 572-581
62. Immke, D. C., and McCleskey, E. W. (2001) Nat. Neurosci. 4(9), 869-870
63. Price, M. P., Thompson, R. J., Eshcol, J. O., Wemnie, J. A., and Benson, C. J. (2004) J. Biol. Chem. 279(51), 53886-53891
FIGURE LEGENDS

Figure 1. The cytosolic C-terminus of ASIC1a contains a putative α-actinin binding site.
Analysis of the ASIC1 C-terminus revealed an amino acid motif that is present in α-actinin-binding cation channels. The gray box highlights the conserved motif in the primary sequence of the mouse ASIC1a cytosolic C-terminus.

Figure 2. α-Actinin and ASIC1a interacted in a heterologous expression system.
Western blots showing isoform-specific interactions between α-actinins and ASIC1a. Because the localization and function of α-actinin is not affected by GFP-tags (45-47), we co-expressed EGFP-tagged isoforms of α-actinin-1, -2, -3 or -4 with ASIC1a in COS-7 cells. For immunoprecipitation, either anti-ASIC1a (top panels) or anti-GFP (middle panels) antibodies were used. Western blots were probed with anti-GFP or anti-ASIC1a antibodies as indicated. Note that anti-ASIC1a antibody co-precipitated EGFP-tagged α-actinin-1 and α-actinin-4 and conversely, anti-GFP antibody co-immunoprecipitated ASIC1a with α-actinin-1 and α-actinin-4. Lower panel shows western blots of total cell lysates (2% of total lysate) used for co-immunoprecipitation experiments.

Figure 3. α-Actinin and ASIC1amut did not interact.
COS-7 cells were transfected with ASIC1a or ASIC1amut alone or together with EGFP-labelled α-actinin-4. For immunoprecipitation, either anti-ASIC1a (upper panels) or anti-GFP (middle panels) antibodies were used (IgG indicates non-specific mouse or rabbit IgG used as a control). Western blots were probed with either anti-ASIC1a or anti-GFP antibody. Note that wild-type ASIC1a, but not ASIC1amut co-immunoprecipitated with α-actinin-GFP. Conversely, α-actinin-GFP co-immunoprecipitated with wild-type ASIC1a, but not ASIC1amut. Lower panels show total cell lysates (2% of total lysate) used for co-immunoprecipitation experiments.

Figure 4. α-Actinin did not alter cell surface expression of ASIC1a.
A) CHO cells were transfected with HA-ASIC1a and either GFP (as a control), α-actinin-4, α-actinin-1 or rod-actinin. After 48h, surface proteins were biotinylated and pulled down with Neutravidin beads. The blot was with anti-HA and anti-tubulin antibodies. Lanes with total protein were loaded with 20% of the cell lysate used for neutravidin binding. B) Quantification of ASIC1a protein surface:total ratio (n = 6 from 3 separate experiments). There were no significant differences between experimental groups.

Figure 5. α-Actinin regulated ASIC1a acid-evoked current in CHO cells.
A) Representative currents evoked by application of pH 5.0 to CHO cells expressing ASIC1a or ASIC1amut alone or co-expressing ASIC1a with α-actinin-1, α-actinin-4, or rod-actinin. B) Mean current densities evoked by pH 5.0 superfusion of CHO cells expressing the indicated constructs. Data are normalized to currents obtained in CHO cells expressing wild-type ASIC1a alone. n = 17-48. Mean H+-gated current densities in CHO cells expressing ASIC1amut were not affected by co-expression with α-actinin-1, α-
Figure 6. α-Actinin modulated pH-sensitivity of ASIC1a current in CHO cells. A) pH-sensitivity of ASIC1a expressed alone in CHO cells (●) or ASIC1a co-expressed with either α-actinin-4 (Δ) or rod-actinin (□). α-Actinin-4 increased pH-sensitivity of ASIC1a, whereas rod-actinin decreased pH-sensitivity (* p< 0.05, n = 3-16 for each data point). “I” indicates peak current amplitude at given pH and “I_pH5.0” indicates peak current amplitude at pH 5.0. B) ASIC1a_mut (■, n = 6-13) had a reduced pH-sensitivity compared to wild-type ASIC1a (●, n = 6) (* p< 0.05). Note that neither co-expressing rod-actinin with ASIC1a_mut (□, n = 6-13) nor coexpressing α-actinin-4 with ASIC1a_mut (Δ, n = 4-13) altered the pH-sensitivity of ASIC1a_mut.

Figure 7. Effect of α-actinin on desensitization of ASIC1a and ASIC1a_mut currents. Data are time constants of desensitization (τ_des) of currents evoked by pH 5.0. τ_des of acid-activated current in CHO cells expressing ASIC1a_mut were not affected by co-expression of α-actinin-1, α-actinin-4 or rod-actinin. n = 16-43 for currents with ASIC1a and the α-actinin variants and n = 6-15 for currents with ASIC1a_mut and the α-actinin variants. * p< 0.01 compared to ASIC1a alone (left) or ASIC1a_mut (right). ** p< 0.05 compared to ASIC1a.

Figure 8. α-Actinin accelerates recovery from desensitization for ASIC1a currents. Acid-activated currents were desensitized by pH 6.0 application for 60 s, followed by application of pH 7.4 for the indicated length of time (5 to 40 s). Then, a second pH 6.0 application was used to assess recovery of ASIC1a current. A) Representative tracings showing recovery from desensitization during 5 s and 20 s at pH 7.4 in CHO cells expressing ASIC1a alone. B) Percentage recovery of current from desensitization in response to pH 6.0 application for 60 s. Data were compared to currents of the first pH 6.0 application. ASIC1a ●, n = 4-9; ASIC1a + rod-actinin Δ, n = 5-10; ASIC1a + α-actinin-4 ◊, n = 4-6; ASIC1a_mut □, n = 4-5. * p< 0.05 compared to ASIC1a alone.

Figure 9. α-Actinin associated with ASIC1a in the brain.
A) Western blot of α-actinin and ASIC1a co-immunoprecipitated from brain lysates. For immunoprecipitation anti-ASIC1a or anti-α-actinin antibodies (BM-75.2, which recognizes all four actinin-isoforms) were used. Western blots were probed with anti-α-actinin antibody (upper panels) or anti-ASIC1a antibody (lower panels). Note that anti-ASIC1a antibody co-precipitated α-actinin, and conversely, anti-α-actinin antibody co-precipitated ASIC1a. Right panels show blots of total cell lysates used for co-immunoprecipitation experiments and were probed with anti-ASIC1a and anti-α-actinin antibody. B) Brain lysates from wild-type and ASIC1a-/- mice (5) were immunoprecipitated with anti-ASIC1a or anti-α-actinin antibody as indicated. The immunoprecipitates were probed with ASIC1a antibody.
Figure 10. α-Actinin regulated H⁺-gated current in hippocampal neurons.

A) Rat hippocampal neurons were tested for the expression of endogenous α-actinins. RT-PCR with isoform-specific primer pairs revealed expression of α-actinin-1, -2 and -4, whereas transcription of α-actinin-3 was not detected. Muscle from the same donor contained transcripts for all four α-actinin isoforms including α-actinin-3. In negative control experiments (-RT) reverse transcriptase was omitted, but the specific primers pairs for all four α-actinin isoforms were present.

B) To assess the efficacy and specificity of siRNA, CHO cells were co-transfected with EGFP-tagged α-actinin-1,-2,-3 or -4 and isoform-specific siRNAs. Western blot was probed with either anti-GFP or anti-tubulin antibody. Each of the four siRNAs attenuated expression of its corresponding α-actinin isoform.

C-F) Reduction of endogenous α-actinin affected properties of acid-activated current in hippocampal neurons. For controls, neurons were transfected either with vector encoding dsRed or siRNA directed against α-actinin-3.

C) Current density was increased by simultaneous knockdown of α-actinin-1, -2 and -4 (n = 23-47, *p <0.02).

D) pH-sensitivity was decreased by knockdown of endogenous α-actinin-1,2,4 ■; pH₅₀ = 6.16, n = 9-36, *p <0.05) compared to controls (dsRed ○, pH₅₀ = 6.30, n = 7-21 and RNAi to α-actinin-3 Δ, pH₅₀ = 6.36, n = 17-28). I indicates peak current amplitude at given pH, and I₉₅.⁰ indicates peak current amplitude at pH 5.0. *p < 0.05.

E) Time-dependent recovery of desensitized current evoked by pH 6.0 (RNAi to α-actinin-1,2,4 ■, n = 5-11 and RNAi to α-actinin-3 Δ, n = 3-11, *p <0.05).

F) Time constants of desensitization of currents evoked by pH 6.0 (n = 18-43).
Table 1: α-Actinin and pH-sensitivity of ASIC1a in CHO-cells.

Data are pH values evoking 50% of current compared to current induced by pH 5.0.

| Transfected DNA       | Half-maximal activation (pH<sub>50</sub>) |
|-----------------------|--------------------------------------------|
| ASIC1a                | 6.46 ± 0.09                                |
| ASIC1a + α-actinin-4  | 6.67 ± 0.14                                |
| ASIC1a + rod-actinin  | 6.30 ± 0.07                                |
| ASIC1a<sub>mut</sub>  | 6.28 ± 0.05                                |
| ASIC1a<sub>mut</sub> + α-actinin-4 | 6.22 ± 0.06    |
| ASIC1a<sub>mut</sub> + rod-actinin | 6.29 ± 0.06    |
Fig. 1

NMDA-R1

P2X7

ASIC1a + 1b

...KNLQDRK...

...KSLQDVK...

...LSLDDVK...

NPCESLRHGHPAGMTYAANILPHHPARGTFEDFTC

Fig. 1
Fig. 2
Fig. 3
Fig. 4

A) Table and Western blot showing the expression of ASIC1a and Tubulin with different conditions.

|         | Surface | Total |
|---------|---------|-------|
| HA-ASIC1a: | +       | +     |
| α-actinin: | - 4 1 Rod | - 4 1 Rod |
| GFP:     | + - - - | + - - - |

B) Bar graph showing the surface to total ratio of ASIC1a with different conditions.

- Control
- 4
- 1
- Rod

α-actinin

Fig. 4
Fig. 5

A) 

ASIC1a
ASIC1a + α-actinin-1
ASIC1a + α-actinin-4

pH 5.0

ASIC1a
ASIC1a + rod-actinin
ASIC1a_{mut}

3 nA
10 s

B) 

ASIC1a
ASIC1a_{mut}

[\text{pA/pF}]

control -1 -4 rod-

α-actinin

control -1 -4 rod-

α-actinin

Fig. 5
Fig. 6
Fig. 7
A) 

B) 

Fig. 8
**Fig. 9**

(A) Blot:
- α-actinin

Blot: ASIC1a

IP: IgG mouse, IgG rabbit, ASIC1a, Lysate

(B) Blot: ASIC1a

IP: ASIC1a α-actinin, ASIC1a α-actinin
Fig. 10

A) 

\[ \begin{array}{c|c|c|c|c|}
\alpha\text{-actinin} & \text{RT} & -1 & -2 & -3 \\
\hline
\text{muscle} & & & & \\
\text{hippocampus} & & & & \\
\end{array} \]

B) 

\[ \begin{array}{c|c}
\text{siRNA to} & \alpha\text{-actinin} \\
\hline
\text{control} & \alpha\text{-actinin-1} \\
\text{RNA} & \alpha\text{-actinin-2} \\
\text{RNA} & \alpha\text{-actinin-3} \\
\text{RNA} & \alpha\text{-actinin-4} \\
\end{array} \]

C) 

\[ \text{Recovery of}\ I_{pH6.0}(\%) \]

D) 

\[ \text{Recovery of}\ I_{pH5.0}(\%) \]

E) 

\[ \text{Recovery of}\ I_{pH6.0}(\%) \]

F) 

\[ \tau_{\text{des pH 6.0}}(\text{ms}) \]

by guest on March 24, 2020http://www.jbc.org/Downloaded from
The cytoskeletal protein α-actinin regulates acid-sensing ion channel 1a through a C-terminal interaction
Mikael K. Schnizler, Katrin Schnizler, Xiang-ming Zha, Duane D. Hall, John A. Wemmie, Johannes W. Hell and Michael J. Welsh

J. Biol. Chem. published online November 21, 2008

Access the most updated version of this article at doi: 10.1074/jbc.M805110200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts