Biochemical and Biophysical Research Communications 424 (2012) 76–81

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Homer proteins accelerate Ca^{2+} clearance mediated by the plasma membrane Ca^{2+} pump in hippocampal neurons

Elizabeth J. Salm, Stanley A. Thayer *

Department of Pharmacology, University of Minnesota Medical School, MN, USA

ARTICLE INFO

Article history:
Received 13 June 2012
Available online 22 June 2012

Keywords:
Homer
PMCA
PDZ domain
Ca^{2+} signaling
Hippocampal neuron

ABSTRACT

The plasma membrane Ca^{2+} ATPase (PMCA) is responsible for maintaining basal intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and returning small increases in $[Ca^{2+}]_i$ back to resting levels. The carboxyl terminus of some PMCA splice variants bind Homer proteins; how binding affects PMCA function is unknown. Here, we examined the effects of altered expression of Homer proteins on PMCA-mediated Ca^{2+} clearance from rat hippocampal neurons in culture. The kinetics of PMCA-mediated recovery from the $[Ca^{2+}]_i$ increase evoked by a brief train of action potentials was determined in the soma of single neurons using indo-1-based photometry. Exogenous expression of Homer 1a, Homer 1c or Homer 2a did not affect PMCA function. However, shRNA mediated knockdown of Homer 1 slowed PMCA mediated Ca^{2+} clearance by 28% relative to cells expressing non-silencing shRNA. The slowed recovery rate in cells expressing Homer 1 shRNA was reversed by expression of a short Homer 2 truncation mutant. These results indicate that constitutively expressed Homer proteins tonically stimulate PMCA function in hippocampal neurons. We propose a model in which binding of short or long Homer proteins to the carboxyl terminus of the PMCA stimulates Ca^{2+} clearance rate. PMCA-mediated Ca^{2+} clearance may be stimulated following incorporation of the pump into Homer organized signaling domains and following induction of the Homer 1a immediate early gene.

1. Introduction

In neurons, the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) regulates processes ranging from neurotransmitter release and excitability [1,2] to gene expression and cell death [3]. Thus, neurons have an elaborate system to regulate $[Ca^{2+}]_i$ that includes influx, sequestration and efflux pathways [4,5]. The interplay of these processes shapes the amplitude, duration and subcellular localization of transient increases in $[Ca^{2+}]_i$ to provide specificity to Ca^{2+} signals [6]. There are two mechanisms to extrude Ca^{2+} from the cell, the low affinity Na^+/Ca^{2+} exchanger and the high affinity plasma membrane Ca^{2+} ATPase (PMCA). The PMCA is responsible for maintaining basal $[Ca^{2+}]_i$ and returning small increases in $[Ca^{2+}]_i$ back to resting levels [7,8].

PMCA-mediated Ca^{2+} extrusion can be modulated by calmodulin, kinases and proteases that affect the carboxyl terminus of the pump [9]. The carboxyl tail of the PMCA contains an autoinhibitory domain that binds to intracellular loops of the pump [10]. Treatments that prevent this intramolecular interaction increase the V_{max} and decrease the K_m for Ca^{2+} translocation [11]. Four genes encode PMCA transcripts that can be alternatively spliced to create an estimated 30 different isoforms [9]. Alternative splicing of the carboxyl terminus encodes proteins with unique modulatory properties [9]. The carboxyl terminus of PMCA "b" splice variants contain a PDZ binding domain [12]; these "b" isoforms bind proteins with PDZ domains such as the Homer 1 protein Ania-3 [13].

The Homer family of proteins regulate cell structure and signaling through their ability to form and regulate cellular scaffolds [14]. All Homer isoforms contain an N terminus Ena/vasodilator-stimulated phosphoprotein homology 1 (EVH-1) domain that contains a PDZ domain capable of binding to Homer ligands such as cell surface receptors, other scaffolding proteins and enzymes involved in cell signaling cascades [15]. Long Homer isoforms have a coiled-coil domain on the carboxyl terminus that binds to other long Homer proteins to form a mesh-like scaffold [16]. Short Homer isoforms lack the coiled-coil domain and thus act in a dominant negative manner by binding to Homer ligands displacing them from the Homer scaffold [16]. Homer proteins bind to PMCAs, but how this binding affects PMCA function is not known.

Here, we tested the hypothesis that Homer proteins bind to PMCAs expressed in hippocampal neurons and modulate pump function. Knockdown of Homer 1 (H1) slowed PMCA mediated Ca^{2+} clearance in a manner reversed by expression of a short

* Corresponding author. Address: Department of Pharmacology, University of Minnesota, 321 Church Street SE, 6-120 Jackson Hall, Minneapolis, MN 55455, USA. Fax: +1 612 625 8408.
E-mail address: sathayer@umn.edu (S.A. Thayer).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.06.069

Homer 2 truncation mutant. These results suggest that binding to Homer proteins is sufficient to accelerate PMCA function.

2. Materials and methods

2.1. Materials

Indo-1 acetoxymethyl ester (AM), Dulbecco's Modified Eagle Medium (DMEM), sera, and Pluronic F-127 were purchased from Invitrogen (Carlsbad, CA). 6-Cyano-7-nitroquinoxaline-2,3,-dione (CNQX) was purchased from Enzo Life Sciences (Farmingdale, NY). All other reagents were purchased from Sigma (St. Louis, MO).

2.2. Primary neuronal cell culture

Rat hippocampal neurons were grown in primary culture as described previously [17] with minor modifications. Maternal rats were euthanized by CO_2 inhalation then E17 fetuses removed and killed by decapitation. Hippocampi were dissected and placed in Ca^{2+} and Mg^{2+}-free HEPES-buffered Hanks salt solution (HHSS), pH 7.45. HHSS was composed of the following (in mM): HEPES 20, NaCl 137, $CaCl_2$ 1.3, $MgSO_4$ 0.4, $MgCl_2$ 0.5, KCl 5.0, KH_2PO_4 0.4, Na_2HPO_4 0.6, $NaHCO_3$ 3.0 and glucose 5.6. Cells were dissociated by trituration through flame-narrowed Pasteur pipettes of decreasing aperture in DMEM without glutamine, supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 U mL^{-1} and 100 μg mL^{-1}, respectively). Dissociated cells then were plated at a density of 70,000–100,000 cells/well onto 25 mm round cover glasses that were coated with Matrigel (200 μL, 0.2 mg mL^{-1}) (BD Biosciences, Billerica, MA). Neurons were grown in an atmosphere of 10% CO_2 and 90% air (pH 7.4) at 37 °C, and fed on days 1 and 6 *in vitro* by exchange of 75% of the media with DMEM, supplemented with 10% horse serum and penicillin/streptomycin.

2.3. $[Ca^{2+}]_i$ Photometry

$[Ca^{2+}]_i$ was recorded from the soma of single hippocampal neurons using indo-1-based photometry with previously described instrumentation [8]. Cells were incubated with 4 μM indo-1 AM and 0.04% pluronic acid for 25–30 min at 37 °C in HHSS. After loading with indo-1 the coverslip was washed with dye-free HHSS for 10 min and mounted in a flow-through chamber (flow rate = 1.5 mL/min). The chamber was placed on an inverted epi-fluorescence microscope with a 70× objective (Leitz, NA = 1.15). Indo-1 was excited at 350 nm (10 nm bandpass) and emission detected at 405 (20) and 495 (20) nm with a pair of photomultiplier tubes (Thorn, EMI) operating in photon-counting mode.

$[Ca^{2+}]_i$ was calculated using the equation $[Ca^{2+}]_i = K_d\beta(R - R_{min})/(R_{max} - R)$, where R is 405/495 nm fluorescent intensity ratio. The dissociation constant (K_d) for indo-1 was 250 nM, and β was the ratio of fluorescence emitted at 495 nm and measured in the absence and presence of Ca^{2+}. R_{min}, R_{max}, and β were determined in intact cells by applying 10 μM ionomycin in Ca^{2+}-free buffer (1 mM EGTA) and saturating Ca^{2+} (5 mM Ca^{2+}). Values for R_{min}, R_{max}, and β were 1.30, 10.87, and 3.16, respectively.

All experiments were performed in HHSS with 5 μM cyclopiazonic acid (CPA) and 10 μM CNQX added to block the sarcoplasmic or endoplasmic reticulum Ca^{2+} ATPase (SERCA) and excitatory synaptic transmission, respectively. Extracellular field stimulation was used to evoke action potentials [18]. At 4 min intervals, a train of voltage pulses (2 ms) was applied across platinum electrodes for six seconds at a rate of 2–10 Hz with a Grass S44 stimulator and stimulus isolation unit (Astro-Med).

2.4. DNA constructs and transfection

Expression plasmids for Homer 1a (H1a) and Homer 2a (H2a) were previously described [17]. Expression plasmids for Homer 2 N-terminus (H2N) and Homer 1c (H1c) were kindly provided by Paul F. Worley [19]. Knockdown of H1 was accomplished using shRNA expression vectors from Open Biosystems/Thermo Fisher Scientific (Lafayette, CO). Effective knockdown was accomplished by transfecting with 2 or 3 shRNA constructs for H1 [pLKO.1 vector; sense sequences (1) CCTGTCTATTATAGAAGGAAT, (2) GCATGCAGTTACTGTATCTTA, (3) GACCCGAACACAAAGAAGAA]. To control for non-specific effects, parallel transfections were performed with equivalent amounts of an expression construct encoding non-silencing shRNA (NS-shRNA) (Open Biosystems/Thermo Fisher Scientific). All constructs were co-transfected with an expression plasmid for GFP (pEGFP-C1, Clontech, Mountain View, CA) or RFP (pTagRFP-N, Evrogen, Moscow, Russia). After 48 h, transfected cells were identified by green [excitation = 480, emission = 540] or red fluorescence [excitation = 555, emission = 584].

Cells were transfected between days 10 and 14 *in vitro* using a previously described calcium phosphate protocol [20]. Briefly, hippocampal cultures were incubated for 20 min in DMEM supplemented with 1 mM kynurenic acid, 10 mM $MgCl_2$, and 5 mM HEPES, to reduce neurotoxicity. A DNA/calcium phosphate precipitate containing 1 μg total plasmid DNA per well was prepared, allowed to form for 30 min at room temperature, and added to the culture. After incubating for 90 min, cells were washed once with DMEM supplemented with $MgCl_2$ and HEPES and then returned to conditioned media, saved at the beginning of the procedure. Transfected neurons were imaged 24–48 h post-transfection. Transfection efficiency ranged from 1% to 5%.

Expression of H1c and H2a were confirmed using immunocytochemistry as previously described [20]. Cells were fixed in ice-cold methanol for 20 min at −20 °C. After blocking in phosphate buffered saline containing 10% bovine serum albumin, cells were incubated with either rabbit anti-Homer 1 (1:250) or goat anti-Homer 2 polyclonal antibodies (1:250, Santa Cruz Biotech, Santa Cruz, CA) followed by secondary antibodies conjugated to tetramethylrhodamine.

2.5. Data analysis

Results are displayed as means ± SE. Monoexponential decay functions were fitted to the data using a nonlinear, least-squares curve fitting algorithm (Origin 8.5 software). Evoked $[Ca^{2+}]_i$ increases greater than 300 nM or less than 200 nM or for which the correlation (r^2) of the fitted curve was less than 0.8 were excluded from analysis. Significance was determined using Student's t test and ANOVA.

3. Results

3.1. H1 knockdown decreases PMCA-mediated $[Ca^{2+}]_i$ clearance

Homer proteins bind to a PDZ-binding domain on the carboxyl tail of b isoforms of the PMCA, but the effects of this interaction on pump function are unknown [13]. To determine the effects of Homer proteins on PMCA function in neurons, we used indo-1-based photometry to measure the rate of PMCA-mediated Ca^{2+} clearance from the soma of rat hippocampal neurons in culture. Three small $[Ca^{2+}]_i$ increases (200–300 nM) were elicited by a brief train of action potentials (6 s, 2–10 Hz) evoked by extracellular field stimulation on 4 min intervals (Fig. 1A). CPA (5 μM) and CNQX (10 μM) were present during all experiments to inhibit SERCA-mediated Ca^{2+} uptake into the endoplasmic reticulum and

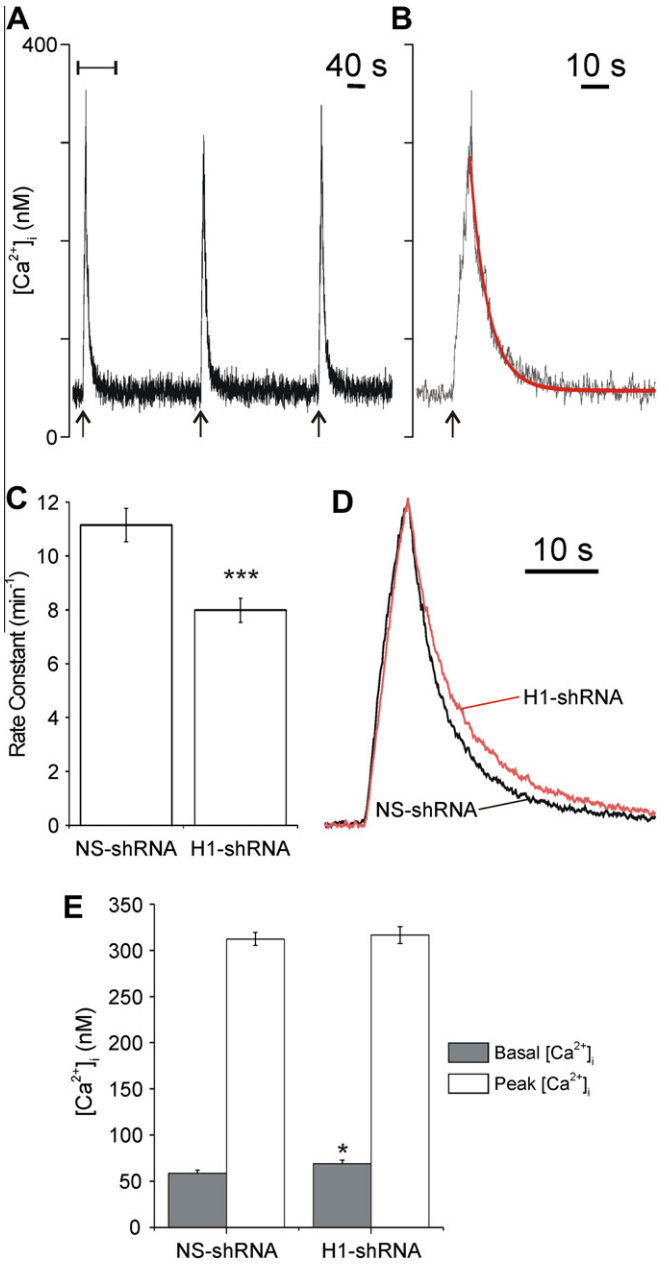

Fig. 1. Homer 1 knockdown decreases PMCA-mediated Ca^{2+} clearance rate. (A) Representative trace shows changes in $[Ca^{2+}]_i$ recorded from the soma of a single rat hippocampal neuron using indo-1-based photometry as described in Section 2. Trains of action potentials were evoked using extracellular field stimulation (6 s, 6 Hz) at the times indicated (↑). CPA (5 μM) and CNQX (10 μM) were present throughout the recording. (B) The first response in A, indicated by the horizontal bar, is shown on an expanded time scale. The recovery of $[Ca^{2+}]_i$ to basal levels was fit with an exponential curve (heavy line). (C) Bar graph shows the average $[Ca^{2+}]_i$ clearance rate in cells expressing H1-shRNA ($n = 21$) and NS-shRNA ($n = 27$). Data are expressed as mean ± SE. $^{***}p < 0.001$ relative to NS-shRNA, Student's t test. (D) Digitally averaged traces show PMCA-mediated $[Ca^{2+}]_i$ recovery kinetics for H1-shRNA ($n = 21$) and NS-shRNA ($n = 27$) expressing cells. (E) Bar graph shows the average basal and peak $[Ca^{2+}]_i$ for cells expressing H1-shRNA and NS-shRNA. $^{*}p < 0.05$ relative to NS-shRNA basal $[Ca^{2+}]_i$, Student's t test. Peak $[Ca^{2+}]_i$ were not significantly different. Data are expressed as mean ± SE.

excitatory synaptic transmission, respectively. We have previously shown that $[Ca^{2+}]_i$ transients of this amplitude in the presence of CPA return to basal levels by a process mediated by PMCAs and are well described by a mono-exponential process (Fig. 1B) [7]. Thus, we used the average rate constant for recovery ($k = 1/\tau$) from 3 responses as an index of PMCA function.

The effects of Homer proteins on PMCA function were evaluated using a knockdown approach. Neurons were transfected with expression vectors for 3 shRNA constructs directed to H1 message (H1-shRNA) or a non-silencing construct (NS-shRNA). Cells were co-transfected with an expression vector for RFP or GFP to enable identification of transfected neurons. Thirty-six hours after transfection, PMCA function was measured in fluorescent cells (Fig. 1C) expressing H1-shRNA or NS-shRNA. Cells expressing H1-shRNA cleared $[Ca^{2+}]_i$ at a rate of $8.0 \pm 0.4\ min^{-1}$, a 28% decrease relative to cells expressing NS-shRNA ($p < 0.001$) which recovered to basal $[Ca^{2+}]_i$ at a rate of $11.0 \pm 0.5\ min^{-1}$ (Fig. 1C and D). Basal $[Ca^{2+}]_i$ in H1-shRNA expressing cells (67 ± 3 nM) was slightly elevated ($p < 0.05$) compared to NS-shRNA expressing cells (58 ± 3 nM; Fig. 1E). Peak $[Ca^{2+}]_i$ in H1-shRNA expressing cells was not significantly different from cells expressing NS-shRNA (Fig. 1E). Thus, decreased expression of H1 slowed PMCA function.

3.2. Over expression of long or short Homer proteins does not alter Ca^{2+} clearance

Long Homer isoforms bind to each other via their coiled coil domains to form scaffolds. In contrast, short Homer isoforms lack a coiled coil domain and thus, bind to Homer ligands but do not anchor them to the scaffold. Indeed, the short isoform H1a acts in a dominant negative manner to uncouple Homer ligands from the scaffold [16]. We next examined the effects of expression of long and short Homer proteins on PMCA function. Neurons were transfected with an expression construct for either a long form, H1c or H2a, or a short form, H1a. Immunocytochemistry confirmed increased H1c and H2a expression in transfected cells. We have previously shown that transfection of hippocampal neurons with the H1a expression vector uncouples H1c-GFP puncta, consistent with its expression and predicted dominant negative effects [17]. The rate of $[Ca^{2+}]_i$ clearance was similar in cells expressing RFP alone, H1c, H2a or H1a (Fig. 2). No significant differences were observed in basal or peak $[Ca^{2+}]_i$ between the groups (data not shown). When considered with the H1 knockdown experiment, these results suggest that Homer binding to PMCA, possibly at the PDZ binding domain on the carboxyl tail, is sufficient to accelerate PMCA kinetics because expression of H1a, which binds to the PMCA and would uncouple it from the Homer scaffold, had no effect of PMCA function.

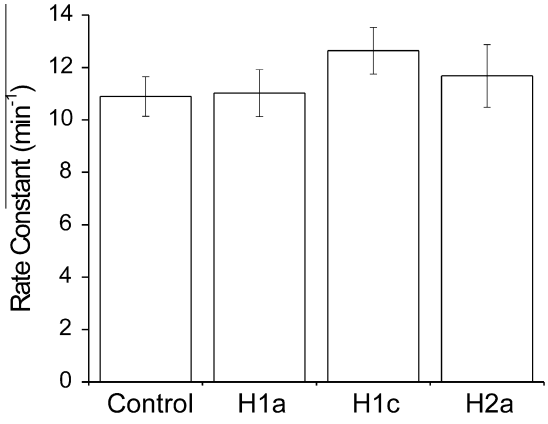

Fig. 2. Over-expression of long or short Homer proteins does not alter Ca^{2+} clearance. Bar graph shows the average $[Ca^{2+}]_i$ clearance rate in cells expressing the fluorescent marker protein (RFP or GFP, $n = 19$) alone or in combination with, H1a ($n = 18$), H1c ($n = 13$) or H2a ($n = 11$). No significant differences were observed in $[Ca^{2+}]_i$ clearance rate. Data are expressed as mean ± SE.

3.3. Short H2N rescues PMCA mediated $[Ca^{2+}]_i$ clearance rate following H1 knockdown

To confirm that the slowed $[Ca^{2+}]_i$ clearance rate in H1-shRNA expressing cells was due to the knockdown of H1 we designed a rescue experiment. Based on sequence analysis, 2 of the 3 H1-shRNA constructs used in the preceding knockdown experiments (Fig. 1) do not significantly react with Homer 2 message. Thus, we first confirmed that transfecting with H1-shRNA constructs 1 and 2 (H1-shRNA$_{1-2}$) was sufficient to slow PMCA mediated Ca^{2+} clearance. As shown in Fig. 3A, control cells expressing H1-shRNA$_{1-2}$ displayed significantly ($p < 0.01$) slowed $[Ca^{2+}]_i$ recovery kinetics ($8.7 \pm 0.7\ min^{-1}$) relative to cells expressing NS-shRNA ($12.1 \pm 0.8\ min^{-1}$). For rescue experiments we co-transfected H1-shRNA$_{1-2}$ with a truncation mutant of H2a. H2N lacks a carboxyl terminal coiled-coil domain and is analogous to H1a in that it binds to Homer ligands but cannot bind to the Homer scaffold [19]. Cells expressing NS-shRNA plus H2N cleared $[Ca^{2+}]_i$ at a rate of $11.8 \pm 1.0\ min^{-1}$ comparable to control NS-shRNA expressing cells (Fig. 3A). Cells expressing H1-shRNA$_{1-2}$ plus H2N cleared $[Ca^{2+}]_i$ at a rate of $10.6 \pm 0.6\ min^{-1}$, which was comparable to cells expressing NS-shRNA plus H2N, and was significantly faster than cells expressing H1-shRNA$_{1-2}$ alone (Fig. 3B; $p < 0.05$). Basal $[Ca^{2+}]_i$ differed between all four cell types while peak $[Ca^{2+}]_i$ was not significantly different ($p < 0.05$; Fig. 3C). H2N rescued PMCA function following H1 knockdown, confirming the specificity of the H1-shRNA. This result also indicates that Homer binding to PMCA is sufficient to accelerate Ca^{2+} clearance and that coupling to the Homer scaffold is not necessary for Homer mediated acceleration of Ca^{2+} clearance.

4. Discussion

PMCA mediated Ca^{2+} clearance from hippocampal neurons was accelerated in the presence of Homer proteins. Knockdown of H1 slowed the Ca^{2+} clearance rate in a manner rescued by expression of H2N. However, expression of exogenous long or short Homer proteins failed to affect PMCA function. Thus, we conclude that constitutively expressed Homer proteins tonically stimulate PMCA function. Because H2N lacks a coiled coil domain, the rescue experiment suggests that Homer binding to the PMCA is sufficient to stimulate Ca^{2+} clearance rate and that integrating into the Homer scaffold is not required for the change in activity.

Homer proteins bind to the PDZ binding domain on the carboxyl terminus of PMCA "b" splice variants [13]. Binding of Ca^{2+} calmodulin to the carboxyl terminus stimulates PMCA function by preventing an intramolecular interaction of the carboxyl terminal autoinhibitory domain with a cytoplasmic loop of the PMCA. Thus, our results are consistent with a model (Fig. 4) in which Homer proteins bind to the carboxyl terminus of the PMCA preventing intramolecular autoinhibition, resulting in accelerated Ca^{2+} pumping. An alternative explanation for slowed PMCA mediated Ca^{2+} clearance following H1 knockdown is reduced PMCA cell surface expression resulting from impaired trafficking in the absence of H1. Reduced trafficking of PMCAs to the cell surface has been described following reduced expression of Na+/H+ exchanger regulatory factor 2 (NHERF-2), a protein that binds to the PDZ binding domain on the carboxyl terminus of PMCA and forms a complex with PMCA at the plasma membrane [21]. Our data do not support membrane recruitment as a mechanism for Homer induced acceleration of PMCA function because the short Homer proteins, which do not join the Homer scaffold, accelerated PMCA function in the H1-shRNA expressing cells (H2N in Fig. 3) and failed to exert a dominant negative effect in wild type cells (H1a in Fig. 2). A role for Homer proteins in PMCA trafficking has not been demonstrated although, Homer proteins affect the trafficking of other membrane proteins such as metabotropic glutamate receptors (mGluRs) [22].

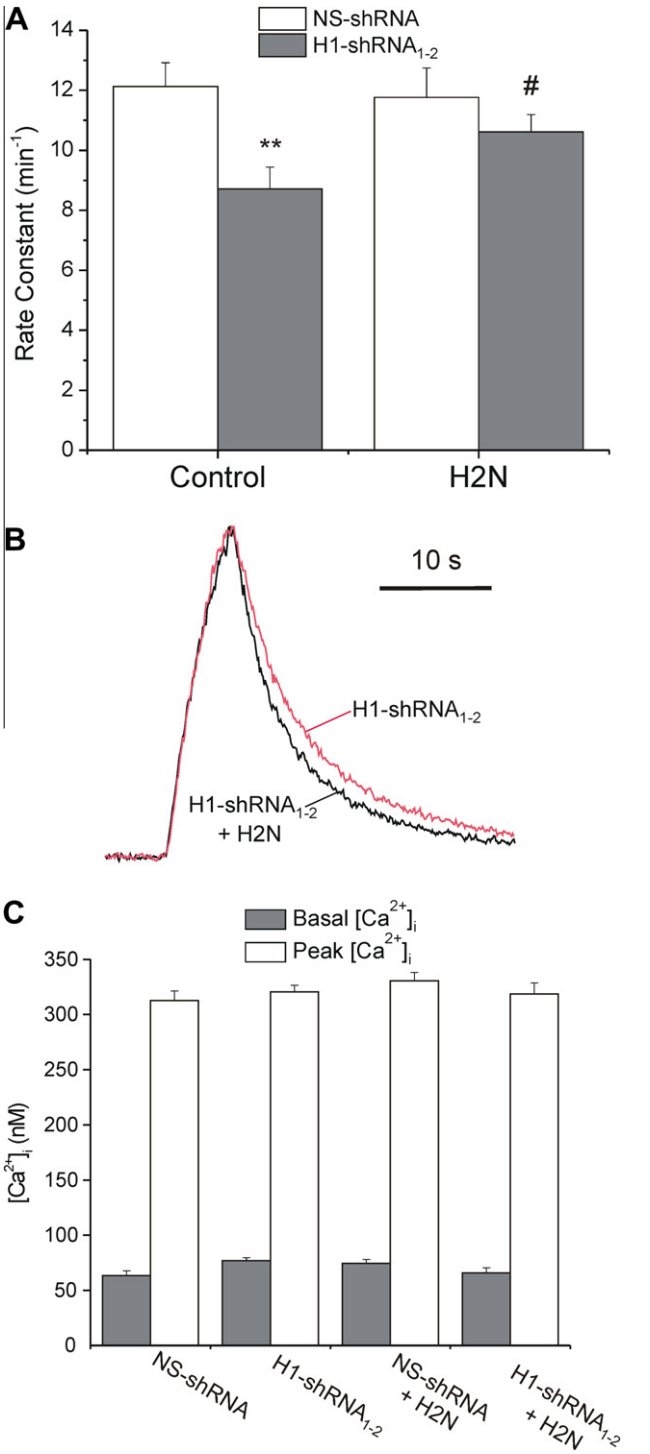

Fig. 3. Short Homer 2 N-terminus rescued $[Ca^{2+}]_i$ clearance rate in H1-shRNA$_{1-2}$ knockdown cells. (A) Bar graph shows the $[Ca^{2+}]_i$ clearance rate in cells expressing NS-shRNA (open bars) or H1-shRNA$_{1-2}$ (solid bars). Cells were transfected with expression vectors for GFP (control) or GFP with H2N as indicated. Data ($n \geqslant 17$) are expressed as mean ± SE. $^{**}p < 0.01$ relative to control NS-shRNA; $^{\#}p < 0.05$ relative to control H1-shRNA$_{1-2}$, Student's t test. (B) Digitally averaged traces show PMCA-mediated $[Ca^{2+}]_i$ recovery kinetics for H1-shRNA$_{1-2}$ ($n = 18$) and H1-shRNA$_{1-2}$ + H2 N ($n = 17$) expressing cells. (C) Bar graph shows the average basal and peak $[Ca^{2+}]_i$ for cells expressing the indicated constructs. ANOVA indicated that mean basal $[Ca^{2+}]_i$ was significantly different, but Tukey's individual mean comparison test did not reach significance ($p < 0.05$). Peak $[Ca^{2+}]_i$ were not significantly different. Data are expressed as mean ± SE.

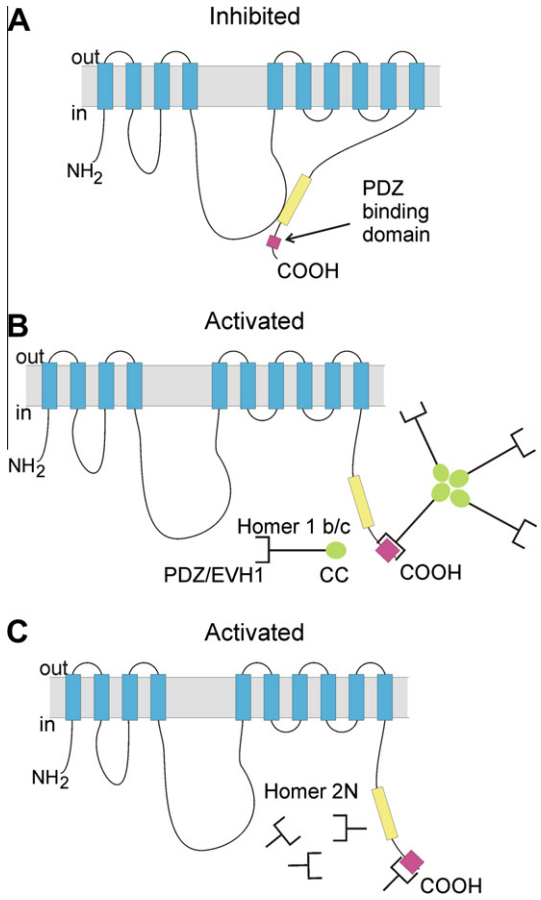

Fig. 4. Schematic shows hypothetical interaction between Homer proteins and PMCA. (A) PMCA in the absence of Homer proteins or other activators is auto-inhibited by the binding of the carboxyl terminus to intracellular domains. (B) Auto-inhibition of PMCA is prevented by the binding of long Homer proteins to the PDZ binding domain, incorporating PMCA into a scaffold. (C) Short Homer proteins prevent auto-inhibition of PMCA without forming a scaffold.

Homer 1b, a long form, reduced cell surface expression by retaining mGluR in the endoplasmic reticulum and H1a, a short form, reversed this effect. Our results in which exogenous expression of short or long Homer proteins failed to affect PMCA function (Fig. 2) are not consistent with this type of trafficking effect for PMCAs. Furthermore, the long Homer proteins are constitutively expressed in hippocampal neurons, which, if long forms retained PMCA in the endoplasmic reticulum, is inconsistent with the slowed PMCA-mediated Ca^{2+} clearance kinetics we observed in H1-shRNA expressing cells. A trafficking effect would predict opposing effects of short versus long Homer proteins; Homer mediated retention in subcellular compartments requires the coiled coil domain and short Homer proteins act in a dominant negative manner to prevent retention. Our results were similar for long and short Homer proteins. Thus, we favor a model in which Homer binding directly modulates PMCA activity.

While binding to the scaffold was not required for Homer-mediated acceleration of PMCA mediated Ca^{2+} clearance, Ca^{2+} pumps bound to the Homer meshwork would be predicted to have fast Ca^{2+} clearance kinetics. Thus, pumps would have increased activity after appropriate targeting to Homer defined locations in the cell. There is precedent for specialized Ca^{2+} signaling assemblies in cerebellar Purkinje cells where Homer 1b/c co-localize with PMCA and inositol trisphosphate receptors in sub-plasma membrane domains [23]. If, as hypothesized, Homer binding to the PMCA interferes with the interaction of the carboxyl terminus with the autoinhibitory domain, then pumps bound to Homers would no longer be sensitive to calmodulin or certain kinases. Thus, Homer binding could force the pump into a sustained activated state occluding modulation by other signals, including Ca^{2+}. This prediction is consistent with the small but significant increase in basal $[Ca^{2+}]_i$ observed in cells expressing H1-shRNA.

In hippocampal neurons, PMCA regulation of $[Ca^{2+}]_i$ affects synaptic plasticity; higher PMCA expression levels in CA2 neurons raise the threshold for the induction of long term potentiation (LTP) relative to CA1 or CA3 neurons [24]. It is not presently known whether "b" splice variants are participating in these functions although "b" variants for all four PMCA transcripts are expressed early in hippocampal development with expression of the "a" variants increasing during maturation [25]. Homer proteins exert strong effects on several signaling pathways in synaptic spines and H1a has been proposed to act as a synaptic tag to modulate synaptic strength [26]. Perhaps Homer modulation of PMCA function is part of a coordinated change in Ca^{2+} signaling properties that sets the threshold for Ca^{2+} dependent synaptic plasticity.

In conclusion, we have demonstrated that Homer proteins accelerate PMCA mediated Ca^{2+} clearance in hippocampal neurons. These results provide a functional outcome for previously described PMCA binding to Homer proteins. The finding that Homer proteins modulate Ca^{2+} efflux rate compliments previous work describing effects on Ca^{2+} influx and release [27]. We suggest that incorporation of PMCA into multi-protein signaling cascades organized by Homer proteins will directly stimulate pump function in addition to bringing the PMCA into close apposition to a Ca^{2+} source. Furthermore, LTP induction and exposure to drugs of abuse alter the expression of Homer proteins, suggesting that changes in $[Ca^{2+}]_i$ clearance might account for some of the changes in cell signaling associated with these stimuli [28,29].

Acknowledgments

The National Science Foundation [Grant IOS0814549] and the National Institute on Drug Abuse [Grants DA07304 and DA11806] supported this work.

References

[1] E. Neher, T. Sakaba, Multiple roles of calcium ions in the regulation of neurotransmitter release, Neuron 59 (2008) 861–872.
[2] T.D. Gover, T.H. Moreira, D. Weinreich, Role of calcium in regulating primary sensory neuronal excitability, Handb. Exp. Pharmacol. (2009) 563–587.
[3] A.M. Oliveira, H. Bading, Calcium signaling in cognition and aging-dependent cognitive decline, Biofactors 37 (2011) 168–174.
[4] C. Grienberger, A. Konnerth, Imaging calcium in neurons, Neuron 73 (2012) 862–885.
[5] S.A. Thayer, Y.M. Usachev, W.J. Pottorf, Modulating Ca^{2+} clearance from neurons, Front. Biosci. 7 (2002) D1255–D1279.
[6] D.D. Friel, H.J. Chiel, Calcium dynamics: analyzing the Ca^{2+} regulatory network in intact cells, Trends Neurosci. 31 (2008) 8–19.
[7] W.J. Pottorf, T.M. Johanns, S.M. Derrington, E.E. Strehler, A. Enyedi, S.A. Thayer, Glutamate-induced protease-mediated loss of plasma membrane Ca pump activity in rat hippocampal neurons, J. Neurochem. 98 (2006) 1646–1656.
[8] J.L. Werth, Y.M. Usachev, S.A. Thayer, Modulation of calcium efflux from cultured rat dorsal root ganglion neurons, J. Neurosci. 16 (1996) 1008–1015.
[9] E.E. Strehler, A.G. Filoteo, J.T. Penniston, A.J. Caride, Plasma-membrane Ca(2+) pumps: structural diversity as the basis for functional versatility, Biochem. Soc. Trans. 35 (2007) 919–922.
[10] A. Enyedi, A.G. Filoteo, G. Gardos, J.T. Penniston, Calmodulin-binding domains from isozymes of the plasma membrane Ca^{2+} pump have different regulatory properties, J. Biol. Chem. 266 (1991) 8952–8956.
[11] E. Carafoli, T. Stauffer, The plasma membrane calcium pump - functional domains, regulation of the activity, and tissue specificity of isoform expression, J. Neurobiol. 25 (1994) 312–324.
[12] E. Kim, S.J. DeMarco, S.M. Marfatia, A.H. Chishti, M. Sheng, E.E. Strehler, Plasma membrane Ca^{2+} ATPase isoform 4b binds to membrane-associated guanylate kinase (MAGUK) proteins via their PDZ (Psd-95/Dlg/Zo-1) domains, J. Biol. Chem. 273 (1998) 1591–1595.

[13] V. Sgambato-Faure, Y. Xiong, J.D. Berke, S.E. Hyman, E.E. Strehler, The Homer-1 protein Ania-3 interacts with the plasma membrane calcium pump, Biochem. Biophys. Res. Commun. 343 (2006) 630–637.

[14] M.U. Ehrengruber, A. Kato, K. Inokuchi, S. Hennou, Homer/Vesl proteins and their roles in CNS neurons, Mol. Neurobiol. 29 (2004) 213–227.

[15] P.R. Brakeman, A.A. Lanahan, R. O'Brien, K. Roche, C.A. Barnes, R.L. Huganir, P.F. Worley, Homer: a protein that selectively binds metabotropic glutamate receptors, Nature 386 (1997) 284–288.

[16] M.K. Hayashi, C. Tang, C. Verpelli, R. Narayanan, M.H. Stearns, R.-M. Xu, H. Li, C. Sala, Y. Hayashi, The postsynaptic density proteins Homer and Shank form a polymeric network structure, Cell 137 (2009) 159–171.

[17] A.M. Roloff, G.R. Anderson, K.A. Martemyanov, S.A. Thayer, Homer 1a gates the induction mechanism for endocannabinoid-mediated synaptic plasticity, J. Neurosci. 30 (2010) 3072–3081.

[18] T.M. Piser, R.A. Lampe, R.A. Keith, S.A. Thayer, W-Grammotoxin blocks action-potential-induced Ca^{2+} influx and whole-cell Ca^{2+} current in rat dorsal root ganglion neurons, Pfluger's Arch. Eur. J. Physiol. 426 (1994) 214–220.

[19] B. Xiao, J.C. Tu, R.S. Petralia, J.P. Yuan, A. Doan, C.D. Breder, A. Ruggiero, A.A. Lanahan, R.J. Wenthold, P.F. Worley, Homer regulates the association of group 1 metabotropic glutamate receptors with multivalent complexes of homer-related, synaptic proteins, Neuron 21 (1998) 707–716.

[20] H.J. Kim, A.H. Shin, S.A. Thayer, Activation of cannabinoid type 2 receptors inhibits HIV-1 envelope glycoprotein gp120-induced synapse loss, Mol. Pharmacol. 80 (2011) 357–366.

[21] W.A. Kruger, C.C. Yun, G.R. Monteith, P. Poronnik, Muscarinic-induced recruitment of plasma membrane Ca^{2+} ATPase involves PSD-95/Dlg/Zo-1 mediated interactions, J. Biol. Chem. 284 (2008) 1820–1830.

[22] K.W. Roche, J.C. Tu, R.S. Petralia, B. Xiao, R.J. Wenthold, P.F. Worley, Homer 1b regulates the trafficking of group I metabotropic glutamate receptors, J. Biol. Chem. 274 (1999) 25953–25957.

[23] D. Sandona, A. Scolari, K. Mikoshiba, P. Volpe, Subcellular distribution of Homer 1b/c in relation to endoplasmic reticulum and plasma membrane proteins in Purkinje neurons, Neurochem. Res. 28 (2003) 1151–1158.

[24] S.B. Simons, Y. Escobedo, R. Yasuda, S.M. Dudek, Regional differences in hippocampal calcium handling provide a cellular mechanism for limiting plasticity, Proc. Natl. Acad. Sci. USA 106 (2009) 14080–14084.

[25] S.N. Kip, N.W. Gray, A. Burette, A. Canbay, R.J. Weinberg, E.E. Strehler, Changes in the expression of plasma membrane calcium extrusion systems during the maturation of hippocampal neurons, Hippocampus 16 (2006) 20–34.

[26] D. Okada, F. Ozawa, K. Inokuchi, Input-specific spine entry of soma-derived Vesl-1S protein conforms to synaptic tagging, Science 324 (2009) 904–909.

[27] P.F. Worley, W. Zeng, G. Huang, J.Y. Kim, D.M. Shin, M.S. Kim, J.P. Yuan, K. Kiselyov, S. Muallem, Homer proteins in Ca^{2+} signaling by excitable and non-excitable cells, Cell Calcium 42 (2007) 363–371.

[28] A. Kato, F. Ozawa, Y. Saitoh, K. Hirai, K. Inokuchi, Vesl, a gene encoding Vasp/Ena family related protein, is upregulated during seizure, long-term potentiation and synaptogenesis, FEBS Lett. 412 (1997) 183–189.

[29] K.K. Szumlinski, A.W. Ary, K.D. Lominac, Homers regulate drug-induced neuroplasticity: implications for addiction, Biochem. Pharmacol. 75 (2008) 112–133.