Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 316 (2004) 189–194
BBRC
www.elsevier.com/locate/ybbrc

AKAP79 increases the functional expression of skeletal muscle Ca^{2+} channels in *Xenopus* oocytes

Consuelo Plata,[a,b] Juan Escamilla,[a] Elba Carrillo,[a] José M. Galindo,[a] Gerardo Gamba,[b] María C. García,[a] and Jorge A. Sánchez[a,*]

[a] *Department of Pharmacology, Centro de Investigación y de Estudios Avanzados del I.P.N., México D.F. 07360, Mexico*
[b] *Unidad de Fisiología Molecular, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán e Instituto de Investigaciones Biomédicas, UNAM. Tlalpan 14000 México D.F., Mexico*

Received 6 February 2004

Abstract

The actions of the kinase A anchoring protein, AKAP79, a key element in the regulation of the cardiac L-type Ca^{2+} channel, were assessed on skeletal muscle Ca^{2+} channels expressed in *Xenopus* oocytes. The channels were reconstituted by expressing the pore forming α_{1s} subunit and its accessory subunits, α_2-δ, β, and γ. We report, for the first time, that peak Ca^{2+} channel currents are greatly increased (3.5-fold) by AKAP79 when co-expressed with the truncated form of the α_{1s} subunit. Immunoblots revealed that the increase in current amplitude is not accompanied by a corresponding increase in the membrane levels of the α_{1s} subunit. This suggests that AKAP79 does not increase the trafficking of the channel. In addition, we show that the transcript of AKAP150, the rat ortholog of the human AKAP79, is expressed in rat skeletal muscle and propose that AKAP79/150 modulates Ca^{2+} channel function.

Keywords: Calcium channel subunit; Skeletal muscle fiber; AKAP; AKAP79; *Xenopus* oocytes; L-type currents; Channel expression; Cav1.1 channels

The dihydropyridine receptors of skeletal muscle play an essential role as the voltage sensors that link excitation with contraction [1–4]. They also function as voltage-dependent Ca^{2+} channels, generating L-type currents [4,5]. Channel activity is influenced by regulatory factors that act within a time frame of seconds or minutes like second messengers and accessory subunits [6–9]. An important regulator of L-type channel activity is protein kinase A, which phosphorylates L-type channels in heart and skeletal muscle [8,10–12]. The specificity and speed of these regulations require a close proximity among the regulatory components that interact with membrane receptors and intracellular molecules. In this regard, scaffolding proteins play a key role. PKA kinase is anchored near L-type channels of skeletal muscle by the kinase anchoring protein AKAP15 [13] while it is anchored in neurons by AKAP79. In addition to this role, AKAP79 also promotes the incorporation of the Cav1.2 channel, the ubiquitous L-type channel present in heart and neurons, to the surface membrane when co-expressed in *Xenopus* oocytes [14]. In the present experiments, we explored whether AKAP79 also influences channel currents and surface expression of muscle Ca^{2+} channels (Cav1.1 channels, see [15]) expressed in oocytes, and found that it dramatically increases the amplitude of channel currents. However, no major changes in the levels of the protein present at the surface membrane were found.

Materials and methods

Preparation. Stage V and VI oocytes were harvested from egg bearing female *Xenopus laevis* frogs under anesthesia with tricaine (0.17%) and incubated for 1 h in the Ca^{2+}-free frog Ringer ND96 containing (in mM): 96 NaCl, 2 KCl, 1 $MgCl_2$, and 5 Hepes/Tris, pH 7.4, plus 2 mg/ml collagenase B (Sigma). Oocytes were then washed four times in a ND96 solution also containing 1.8 mM $CaCl_2$. Oocytes were defolliculated manually and then stored in the same medium at

* Corresponding author. Fax +52-55-55-77-70-90.
E-mail address: jsanchez@mail.cinvestav.mx (J.A. Sánchez).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.02.035

16 °C supplemented with 2.5 mM sodium pyruvate and 50 μg/ml gentamicin (Gibco-BRL) at pH 7.6. Next day oocytes were injected with 50 nl of a mixture of α_{1s} (or $\alpha_{1s\Delta c}$), β_{1b} (or β_{1a}), α_2-δ, and γ cRNA each at a concentration of 0.5 μg/μl. Injection pipettes were fabricated from thin-walled capillary glass (WPInstruments, Sarasota, FL, USA). Oocytes were stored at 16 °C for 11–12 days before recordings.

The external solution employed to record L-type currents contained (mM): 40 $Ba(OH)_2$, 50 NaOH, 1 KOH, 0.5 niflumic acid, 0.1 EGTA, and 5 Hepes at pH 7.4, adjusted with methanesulfonic acid.

Dissociated muscle fibers from BALB/c mice (age ~9 weeks) were used; mice were killed by rapid dislocation of the neck, after which the flexor digitorum brevis muscles (fdb) of the hindlimb were isolated and incubated at 34 °C for 60 min in a Ca^{2+}–Mg^{2+}-free Tyrode solution containing (mM): NaCl, 146; KCl, 5; glucose, 11; and Hepes, 10; at pH 7.4 plus collagenase (0.5 mg/ml, Sigma type lV) and 10% fetal calf serum (Gibco-Invitrogen). Isolated fibers were obtained by gently triturating the enzyme-treated muscles through a fire-polished Pasteur pipette. The muscles were then rinsed with collagenase-free Tyrode solution supplemented with $CaCl_2$, 2 mM; $MgCl_2$, 1 mM. Experiments were performed following the guidelines laid down by the Local Animal Care Committee. The external solution employed to record Ca^{2+} currents contained (mM): 2 $MgCl_2$, 10 Ca^{2+}, 140 TEA^+, and methanesulfonate as anion. The pipette solution contained (mM): 140 Cs-aspartate, 5 $MgCl_2$, and 10 EGTA. Extracellular and intracellular solutions were buffered with Hepes (10 mM) at pH 7.2 and 7.1, respectively.

In vitro synthesis of RNA. The rabbit $\alpha_{1s\Delta c}$, (lacking base pairs 5320–5844, corresponding to amino acids 1698–1893 in the C-terminus region), the rat brain β_{1b} and the rabbit γ, and rabbit α_2-δ Ca^{2+} channel subunit cDNAs were a gift from Dr. Steve Cannon. The rabbit full length α_{1s} cDNA and the mouse β_{1a} were kindly provided by Dr. Edward Perez-Reyes and Dr. Patricia Powers, respectively. The AKAP79 cDNA was provided by Dr. WenHui Wang.

For in vitro synthesis of RNA (run-off), the plasmids containing the Ca^{2+} channel subunits were linearized using the appropriate restriction enzymes. Capped cRNA coding for all subunits was synthesized using the mMessage Machine kit (Ambion, Austin, TX, USA) and cleaned using the RNAid purification kit (Bio101, Vista, CA, USA).

RT-PCR. Total cellular RNA was extracted from *gastrocnemius* muscle bundles of adult Wistar rats as in [16]. Rats were killed by a lethal injection of pentobarbital sodium. cDNA was obtained from 5 μg of total RNA, using SuperScript II Reverse Transcriptase (Gibco-BRL) and random hexamers. Five percentage of the reverse transcription reaction volume was used for the PCRs. cDNA was mixed with 50 pmol of each primer in a final volume of 50 μl of 1× PCR buffer that contained: 0.2 mM of each of the dNTPs, 2 mM $MgCl_2$, and 2.5 U Platinum polymerase (Gibco). Following an initial treatment of 3 min at 95 °C, the following cycle was repeated 35 times: 30 s at 95 °C, 30 s at 62 °C, and 40 s at 72 °C. Final extension was 10 min at 72 °C. As a positive control, we used the PCR product derived from rat brain RNA. The oligonucleotide sequence was: AKF 5′-GCA GCC AGT CCT CAG AAA GAG-3′ and AKR 5′-TCC AAA TCG TCT GCC TCT CCC-3′. These primers amplify the region between nucleotides 582 and 988 of rat AKAP150, the ortholog of the human AKAP79. This sequence codes the amino acids 20–155 in the N-terminal region of the protein, involved in protein targeting to the cell membrane [17].

To characterize the amplified transcript, the muscle PCR product was purified and digested with *Sac*l and *Pst*l restriction enzymes. The identity of the product was confirmed by sequencing (Perkin–Elmer, model ABI PRISM 310).

Western blots. Crude oocyte membranes were prepared by homogenizing 40 minced oocytes in 5 vols of 0.3 M sucrose and 10 mM imidazole, at pH 7.4. The following protease inhibitors were used: 200 μM phenylmethylsulfonyl fluoride (PMSF), 1 mM benzamidine, 100 nM aprotinin, and 1 μM pepstatin. The homogenate was centrifuged at 11,000*g* for 10 min and the supernatant was set aside. The pellet was rehomogenized and centrifuged at 14,500*g* for 15 min. The supernatants were collected and centrifuged at 45,000*g* for 90 min to yield crude oocyte membranes. A similar procedure, used to prepare crude membranes, has been previously described [18]. The pellet was resuspended in 0.1 ml of homogenizing medium and loaded into 7.5% sodium dodecyl sulfate–polyacrylamide gels. Protein concentrations were determined as in [19]. The sample volume used in Western blots was adjusted based on the density of the Lipoviteline protein band. The gel proteins were then transferred to a nitrocellulose membrane, probed with the primary antibody (anti-pan α_1 [anti-CP-(1382–1400)], from Alomone Labs, Jerusalem, Israel, at 1:300 dilution), followed by the secondary antibody (anti-rabbit horseradish peroxidase conjugate from Amersham Life Sciences, at 1:2500 dilution) and detected by enhanced chemiluminescence using an ECL detection kit (Amersham). The blots were blocked with 5% nonfat dried milk in phosphate-buffered saline. The density of the $\alpha_{1s\Delta c}$ subunit bands was measured using the KodaKID Image Analysis Software.

Electrophysiological methods. Membrane currents were recorded in oocytes with a two microelectrode voltage clamp amplifier (Axoclamp, Axon Instruments). Pipettes were filled with 3 M KCl and had tip resistances of 0.5–2.5 MΩ.

A patch-clamp amplifier (Axopatch 200A, Axon Instruments) was used for whole-cell recordings [20] from muscle fibers as described by others [21]. Pipettes had resistances of 1–1.2 MΩ.

To minimize mechanical artifacts, intact fibers were incubated during two hours, prior to patch clamp recordings, in 20 μM of the cell permeant Ca^{2+} buffer BAPTA-AM (molecular probes, Inc.), added to the external solution.

Electrophysiological experiments were carried out at room temperature (20–22 °C).

Data collection and pulse protocol. Membrane currents were sampled as detailed elsewhere [22]. To measure activation of L-type channels, command pulses of variable amplitude lasting 1.5 s (in oocyte experiments) or 0.75 s (in muscle fiber experiments) were delivered. Nonlinear currents from muscle fibers were obtained as in [22]. To subtract linear currents in oocytes, currents generated by a +30 mV depolarizing pulse were used.

To measure the time course of activation and decay of Cav1.1 currents, the current data points were fitted to:

$$I = A(1 - \exp(-t/\tau_1))(B - (B - C)\exp(-t/\tau_2)), \quad (1)$$

where A is an amplitude factor, τ_1 and τ_2 are the time constants of activation and decay, respectively, and B and C represent the fractions of channels that can open at the end and at the beginning of the pulse, respectively. A similar equation was used to analyze the kinetic properties of Ca^{2+} channels in muscle [5] except that the power of the activation factor used in the present study was 1. The fitting of Eq. (1) employed a non linear least squares algorithm.

Average values are expressed as means ± SE. To calculate statistical significance, Student's t test was used at the level $p < 0.05$.

Results and discussion

Expression of the skeletal muscle Cav1.1 channel in Xenopus oocytes

Cav1.1 channel currents are recorded in *Xenopus* oocytes when the truncated form of α_{1s}($\alpha_{1s\Delta c}$) is co-expressed with the β_{1b} subunit, an accessory subunit abundantly expressed in neurons but expressed at a low level in skeletal muscle [23]. Currents are also recorded when the full length form of the channel (α_{1s}) is co-expressed with the β_{1b} subunit plus the auxiliary subunits α_2-δ and γ [24]. Our results confirm and extend these observations. Fig. 1A

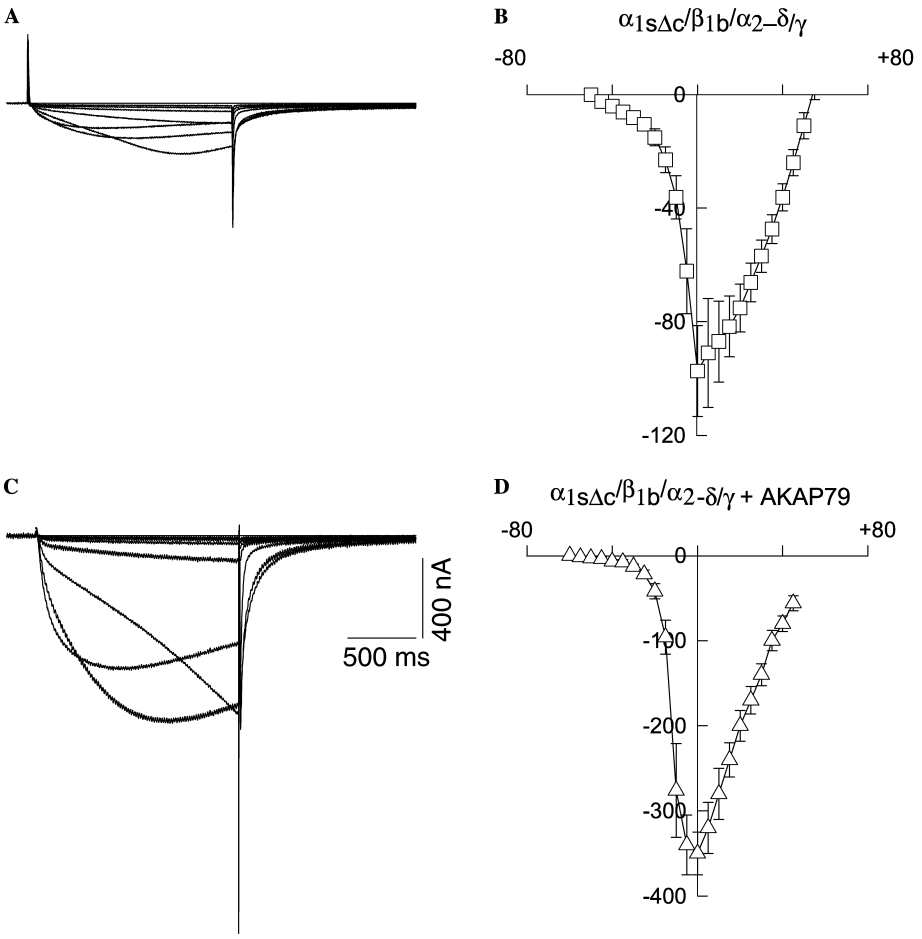

Fig. 1. The effect of AKAP79 on Cav.1.1 currents. The records show superimposed non-linear currents during voltage steps from −60 mV to +10 mV in 10 mV steps. (A) Cav1.1 channel current records from an oocyte expressing the combination: $\alpha_{1s\Delta c}/\beta_{1b}/\alpha_2$-$\delta/\gamma$. (C) Current records from a separate experiment expressing the same combination plus AKAP79. (B,D) Current–voltage relation of peak L-type currents recorded from oocytes expressing the combination shown above each graph. Each point represents average value (±SE) of at least five experiments. Notice that the current scale in (D) is different from that in (B).

illustrates superimposed current records from an oocyte expressing $\alpha_{1s\Delta c}/\beta_{1b}/\alpha_2$-$\delta/\gamma$ subunits. Fig. 1B shows the average current–voltage relation of peak currents. Activation of Cav1.1 currents began at about −40 mV, currents reached a peak value of -97.4 ± 16.1 nA ($n = 11$) close to 0 mV and declined during larger depolarizations with an apparent reversal potential close to +50 mV. The time to peak of Cav1.1 currents during large depolarizations averaged 152.0 ± 10.9 ms ($n = 11$). Significantly smaller and slower currents were measured when the full length α_{1s} subunit was co-expressed with the same accessory subunits. The peak current amplitude averaged -36.6 ± 10.8 nA ($n = 4$) and the time to peak, 680 ± 73.6 ms ($n = 4$). On the other hand, no L-type currents were recorded when the α_{1s} subunit and the muscle β_{1a} subunit (plus α_2-δ and γ subunits) were co-expressed, but distinct currents were observed when the $\alpha_{1s\Delta c}$ subunit was co-expressed with the same accessory subunits. In these experiments the peak current averaged -71.4 ± 13.6 nA ($n = 4$) and their time to peak, 192 ± 22 ms ($n = 4$). Overall, these data indicate that L-type currents are best recorded with the subunit combination: $\alpha_{1s\Delta c}/\beta_{1b}/\alpha_2$-$\delta/\gamma$. It should be noted that the predominant isoform of the α_1 subunit in muscle is the truncated one. It results from posttranslational cleavage in the C-terminal domain [11].

Actions of AKAP79 on Cav1.1 channels

To study the actions of AKAP79 on Cav1.1 channels, we expressed $\alpha_{1s\Delta c}$, β_{1b}, α_2-δ, and γ subunits in *Xenopus* oocytes. The main finding of the present study is that AKAP79 greatly increased the amplitude of $Ca_V1.1$ currents, as shown in Figs. 1C–D. Fig. 1C shows that AKAP79 co-expression increased current amplitude at all potentials (Fig. 1A). Fig. 1D shows the average current–voltage relation from oocytes in which AKAP79 was co-expressed. Currents also peaked at 0 mV as in control experiments (Fig. 1B), but reached a much larger value, -350.1 ± 24.8 nA ($n = 7$), indicating an increase in $Ca_V1.1$ current amplitude of more than

threefold without significant shifts along the voltage axis. A similar increase in the amplitude of L-type currents was observed in *Xenopus* oocytes when AKAP79 is co-expressed with α_{1c}, the principal subunit of Cav1.2 channels [14].

A major property of AKAPs is the binding of PKA and its targeting to the appropriate substrates that are to be phosphorylated [25]. To test whether PKA is involved in the increase in current amplitude produced by expression of AKAP79, we stimulated the PKA cascade with 50 μM forskolin and found that it did not alter the magnitude of the AKAP79 effect. In the presence of forskolin, L-type currents averaged -356 ± 27 nA ($n = 3$) in oocytes that expressed AKAP79 plus $\alpha_{1s\Delta c}/\beta_{1b}/\alpha_2$-$\delta/\gamma$ subunits. Likewise, 8-Br-cAMP was also ineffective. A similar conclusion was reached when AKAP79 was co-expressed with α_{1c} channels [14]. Our results do not entirely rule out changes in the phosphorylation level of Cav1.1 channels since AKAP79 also binds PKC, calmodulin, and the phosphatase calcineurin. The activity of calcineurin is inhibited by AKAP79 [25–27].

AKAP15, a cAMP-dependent anchoring protein that co-immunoprecipitates with the skeletal muscle Ca^{2+} channel [13], is involved in the voltage potentiation of Cav1.1 currents [28]. AKAP15 interacts with the C-terminus domain of the channel, in the region located between residues 1774 and 1821 [28]. Since in the present study we show that AKAP79 modulates channel function of Cav1.1 channels that were reconstituted with $\alpha_{1s\Delta c}$, lacking residues 1698–1893, AKAP79 must have a different target, not necessarily in the Ca^{2+} channel, that remains to be identified. The PP region (residues 744 and 766), a conspicuous proline motif, conserved in several L-type channels, including the muscle Ca^{2+} channel, is essential for the action of AKAP79 on Cav1.2 channels [14], and may also be involved in the regulatory effects of AKAP79 on Cav1.1 channels.

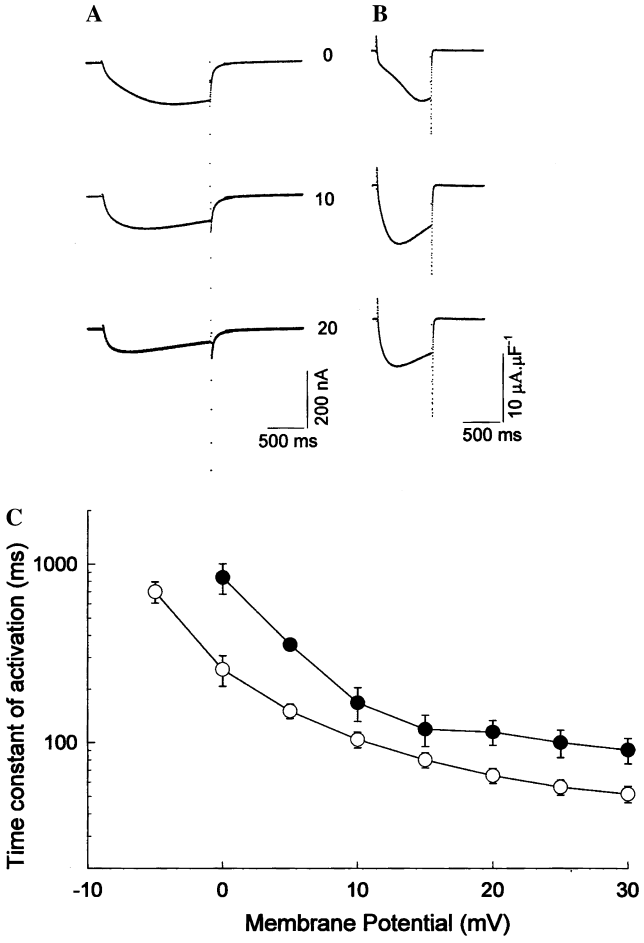

Fig. 2. The activation of L-type currents in oocytes and muscle fibers. The records show non-linear membrane currents during depolarizing pulses to the potentials indicated (in mV). (A) Currents recorded from an oocyte expressing the combination: AKAP79/$\alpha_{1s\Delta c}/\beta_{1b}/\alpha_2$-$\delta/\gamma$. (B) Current records from a mouse skeletal muscle fiber. Notice that the pulse duration in (A) is different from that in (B). (C) The relationship between the activation time constant and membrane potential. (○) Represent average values (±SE) of 5–7 experiments from oocytes expressing the Cav1.1 channel. (●) Represent average values (±SE) from 3–10 mouse skeletal muscles fibers.

Time course of activation of Cav1.1 currents

To compare the kinetic properties of Cav1.1 channels, co-expressed in oocytes with AKAP79, with those of skeletal muscle fibers, currents were recorded in both preparations and were fitted to Eq. (1). Fig. 2A shows Cav1.1 currents recorded at the potentials indicated (in mV) from an oocyte and Fig. 2B from a skeletal muscle fiber. In both cases, current activation and decay depended on the depolarization level during the pulses. The most noticeable difference between oocyte and muscle Cav1.1 currents was the much faster time course of decay of the latter compared with the former. This is expected since Cav1.1 currents in muscle decay quickly by depletion of divalent cations in the lumen of the transverse tubular system overcoming inactivation [29]. Depletion prevented a comparison between inactivation of Cav1.1 currents in oocytes and muscle fibers.

In addition to changes in the rate of decay, there were also differences in the time course of activation, although less pronounced. Fig. 2C shows a semilogarithmic plot of the voltage dependence of the activation time constant. (○) represent average results from oocyte recordings and (●) from muscle fibers. In both cases, activation progressively became faster as the level of depolarization increased. However, the time constant of activation was smaller in oocytes at all potentials. For example, $\tau = 51.8 \pm 5.4$ ms ($n = 7$) at +30 mV in oocytes and $\tau = 91.1 \pm 14.8$ ms ($n = 7$) in skeletal muscle fibers. Several factors may contribute to this difference. The β_1 subunit has dramatic effects on channel kinetics [30] and may not be interacting with $\alpha_{1s\Delta c}$ in oocytes in the same manner as in muscle. Second, currents recorded in muscle

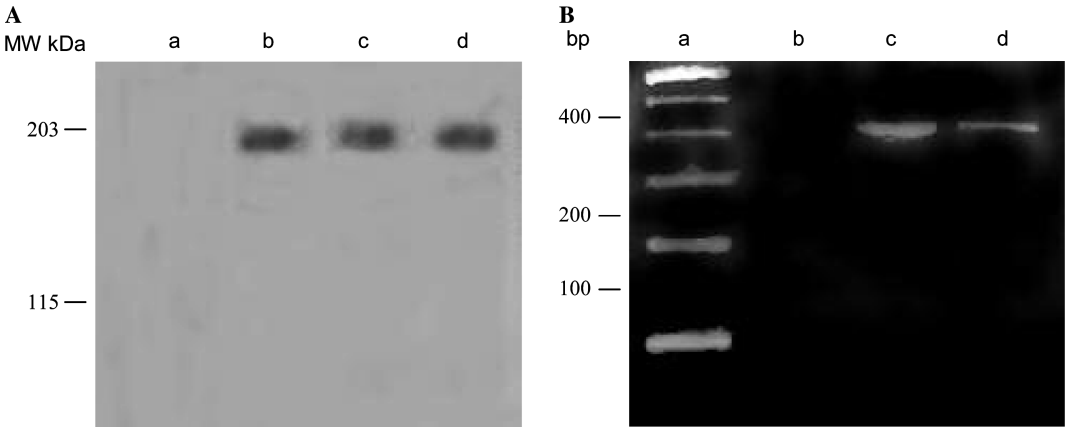

Fig. 3. Expression of Cav1.1 channels at the protein level and detection of AKAP79 transcript. (A) The blot shows that an anti-pan α_1 antibody recognizes a single band at approximately 170 kDa in oocytes that expressed the combination: $\alpha_{1s\Delta c}/\beta_1/\alpha_2$-$\delta/\gamma$. The density of the band did not depend on whether β_{1a} (lane b) or β_{1b} (lane c) subunits were co-expressed. Co-expression with AKAP79 had no effect on $\alpha_{1s\Delta c}$ expression at the protein level (lane d). The band was absent in oocytes injected with water (lane a). (B) RT-PCR, using primers AKF and AKR with RNA from rat brain (lane c), rat skeletal muscle (lane d) or muscle RNA without reverse transcriptase (lane b). Lane a, a 100 base pair DNA ladder.

membrane vesicles also activate quickly, suggesting that other factors present in muscle regulate channel kinetics [22]. Finally, the charge carrier may play a role as in mouse myotubes [31].

Expression of Cav1.1 channels at the protein level

Western blot experiments were performed to determine whether the increase in the amplitude of Cav1.1 channel currents caused by co-expression of AKAP79 is due to an increase in the channel protein content at the membrane level. A threefold increase would be readily detectable in the immunoblot. In fact, this technique reveals small changes in $\alpha_{1s\Delta c}$ in skeletal muscle by long term depolarization [32]. Fig. 3A shows representative results. A distinct band corresponding to $\alpha_{1s\Delta c}$ was detected when this subunit was co-expressed with β_{1a}, α_2-δ, and γ subunits (lane b). A similar expression level (lane c) was observed when the $\alpha_{1s\Delta c}$ subunit was co-expressed with the β_{1b} subunit (plus α_2-δ and γ subunits). A significant membrane expression of the $\alpha_{1s\Delta c}$ subunit when co-expressed with either isoform of the β subunit is consistent with a previous report based on gating charge measurements [24].

When $\alpha_{1s\Delta c}$ (plus the β_{1b}, α_2-δ, and γ subunits) was co-expressed with AKAP79, no changes in the expression level of $\alpha_{1s\Delta c}$ were observed (lane d). Thus, the ratio between the density of the $\alpha_{1s\Delta c}$ band, from oocytes co-expressing AKAP79, and that of the $\alpha_{1s\Delta c}$ band in control experiments, averaged 0.93 ± 0.04 ($n = 3$). This suggests that the increase in current amplitude by AKAP79 cannot be explained by an increase in the trafficking of the $\alpha_{1s\Delta c}$ subunit to the membrane. Instead, the current increase is probably due to an enhancement in the efficacy of DHP receptors to function as Ca^{2+} channels.

Detection of AKAP 79 transcript in skeletal muscle

Our data indicate that AKAP79 greatly increases Cav1.1 currents in *Xenopus* oocytes. If this anchoring protein also regulates Cav1.1 currents in skeletal muscle, it should be expressed in this tissue. To test this possibility, we performed RT-PCR experiments using mRNA from rat skeletal muscle or brain (Fig. 3B) as template and primers AKF and AKR specific for AKAP150, the rat ortholog of the human AKAP79. A PCR product of the expected size (400 bp) was detected in rat skeletal muscle (Fig. 3, lane d). Restriction enzymes revealed products of the expected size: 137 and 269 bp with *Sac*I and 185 and 221 bp with *Pst*I. The PCR product had the expected sequence.

The presence of two distinct anchoring proteins, AKAP15 and AKAP79, in skeletal muscle would not be unprecedented. For example, in hippocampal neurons both AKAP79 [27] and AKAP15 [33] have been identified.

Acknowledgments

The authors thank Drs. E. Perez Reyes, S.C. Cannon, P. Powers, and W. Wang for the gift of Ca^{2+} channel subunits and AKAP cDNAs. We thank A. Hernández and O. Ramirez for technical assistance and S. Zamudio for secretarial work. This work was supported by CONACyT Grant 32055N to JS, Grant 37356N to MG and by NIH Grant (DHHS) 1 R01 HL63903-01A1, and 18 (UC-MEX).

References

[1] E. Ríos, G. Pizarro, Voltage sensor of excitation-contraction coupling in skeletal muscle, Physiol. Rev. 71 (1991) 849–908.
[2] G.D. Lamb, DHP receptors and excitation-contraction coupling, J. Muscle Res. Cell. Motil. 13 (1992) 394–405.

[3] C.L.H. Huang, Intramembrane charge movements in striated muscle, Clarendon Press, Oxford, England, 1993.
[4] W. Melzer, A. Herrmann-Frank, H.C. Luttgau, The role of Ca^{2+} ions in excitation-contraction coupling of skeletal muscle fibres, Biochim. Biophys. Acta 1241 (1995) 59–116.
[5] J.A. Sánchez, E. Stefani, Kinetic properties of calcium channels of twitch muscle fibres of the frog, J. Physiol. 337 (1983) 1–17.
[6] J. Arreola, J. Calvo, M.C. García, J.A. Sanchez, Modulation of calcium channels of twitch skeletal muscle fibres of the frog by adrenaline and cyclic adenosine monophosphate, J. Physiol. 393 (1987) 307–330.
[7] A. Sculptoreanu, T. Scheuer, W.A. Catterall, Voltage-dependent potentiation of L-type Ca^{2+} channels due to phosphorylation by cAMP-dependent protein kinase, Nature 364 (1993) 240–243.
[8] W.A. Catterall, Structure and regulation of voltage-gated Ca^{2+} channels, Annu. Rev. Cell. Dev. Biol. 16 (2000) 521–555.
[9] R. García, E. Carrillo, S. Rebolledo, M.C. García, J.A. Sánchez, The β_{1a} subunit regulates the functional properties of adult frog and mouse L-type Ca^{2+} channels of skeletal muscle, J. Physiol. 545 (2002) 407–419.
[10] B.M. Curtis, W.A. Catterall, Phosphorylation of the calcium antagonist receptor of the voltage-sensitive calcium channel by cAMP-dependent protein kinase, Proc. Natl. Acad. Sci. USA 82 (1985) 2528–2532.
[11] K.S. De Jongh, C. Warner, A.A. Colvin, W.A. Catterall, Characterization of the two size forms of the α1 subunit of skeletal muscle L-type calcium channels, Proc. Natl. Acad. Sci. USA 88 (1991) 10778–10782.
[12] T. Gao, A. Yatani, M.L. Dell'Acqua, H. Sako, S.A. Green, N. Dascal, J.D. Scott, M.M. Hosey, cAMP-dependent regulation of cardiac L-type Ca^{2+} channels requires membrane targeting of PKA and phosphorylation of channel subunits, Neuron 19 (1997) 185–196.
[13] P.C. Gray, V.C. Tibbs, W.A. Catterall, B.J. Murphy, Identification of a 15-kDa cAMP-dependent protein kinase-anchoring protein associated with skeletal muscle L-type calcium channels, J. Biol. Chem. 272 (1997) 6297–6302.
[14] C. Altier, S.J. Dubel, C. Barrere, S.E. Jarvis, S.C. Stotz, R.L. Spaetgens, J.D. Scott, V. Cornet, M. De Waard, G.W. Zamponi, J. Nargeot, E. Bourinet, Trafficking of L-type calcium channels mediated by the postsynaptic scaffolding protein AKAP79, J. Biol. Chem. 277 (2002) 33598–33603.
[15] E.A. Ertel, K.P. Campbell, M.M. Harpold, F. Hofmann, Y. Mori, E. Perez-Reyes, A. Schwartz, T.P. Snutch, T. Tanabe, L. Birnbaumer, R.W. Tsien, W.A. Catterall, Nomenclature of voltage-gated calcium channels, Neuron 25 (2000) 533–535.
[16] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid-guanidinium thiocyanate-phenol-chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[17] M.L. Dell'Acqua, M.C. Faux, J. Thorburn, A. Thorburn, J.D. Scott, Membrane-targeting sequences on AKAP79 bind phosphatidylinositol-4,5-bisphosphate, EMBO J. 17 (1998) 2246–2260.
[18] Y. Péréon, C. Dettbarn, Y. Lu, K.N. Westlund, J.T. Zhang, P. Palade, Dihydropyridine receptor isoform expression in adult rat skeletal muscle, Pflugers Arch. 436 (1998) 309–314.
[19] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[20] O.P. Hamill, A. Marty, E. Neher, B. Sakmann, F.J. Sigworth, Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches, Pflugers Arch. 391 (1981) 85–100.
[21] Z. Wang, M.L. Messie, O. Delbono, Patch-clamp recording of charge movement, Ca^{2+} current, and Ca^{2+} transients in adult skeletal muscle fibers, Biophys. J. 77 (1999) 2709–2716.
[22] J. Camacho, A. Carapia, J. Calvo, M.C. García, J.A. Sánchez, Dihydropyridine-sensitive ion currents and charge movement in vesicles derived from frog skeletal muscle plasma membranes, J. Physiol. (Lond) 520 (1999) 177–186.
[23] D. Ren, L.M. Hall, Functional expression and characterization of skeletal muscle dihydropyridine receptors in *Xenopus* Oocytes, J. Biol. Chem. 272 (1997) 22393–22396.
[24] J.A. Morrill, S.C. Cannon, COOH-terminal truncated α_{1s} subunits conduct current better than full-length dihydropyridine receptors, J. Gen. Physiol. 116 (2000) 341–348.
[25] K. Dodge, J.D. Scott, AKAP79 and the evolution of the AKAP model, FEBS Lett. 476 (2000) 58–61.
[26] D.W. Carr, R.E. Stofko-Hahn, I.D. Fraser, R.D. Cone, J.D. Scott, Localization of the cAMP-dependent protein kinase to the postsynaptic densities by A-kinase anchoring proteins, characterization of AKAP 79, J. Biol. Chem 267 (1992) 16816–16823.
[27] V.M. Coghlan, B.A Perrino, M. Howard, L.K. Langeberg, J.B. Hicks, W.M. Gallatin, J.D. Scott, Association of protein kinase A and protein phosphatase 2B with a common anchoring protein, Science 267 (1995) 108–111.
[28] J.T. Hulme, N. Ahn, S.D. Hauschka, T. Scheuer, W.A. Catterall, A novel leucine zipper targets AKAP15 and cyclic AMP-dependent protein kinase to the C terminus of the skeletal muscle Ca^{2+} channel and modulates its function, J. Biol. Chem. 277 (2002) 4079–4087.
[29] W. Almers, R. Fink, P.T. Palade, Calcium depletion in frog muscle tubules: the decline of calcium current under maintained depolarization, J. Physiol. 312 (1981) 177–207.
[30] A.E. Lacerda, H.S. Kim, P. Ruth, E. Perez-Reyes, V. Flockerzi, F. Hofmann, L. Birnbaumer, A.M. Brown, Normalization of current kinetics by interaction between the α_1 and the β subunits of the skeletal muscle dihydropyridine-sensitive Ca^{2+} channel, Nature 352 (1991) 527–530.
[31] R.T. Dirksen, K.G. Beam, Single calcium channel behavior in native skeletal muscle, J. Gen. Physiol. 105 (1995) 227–247.
[32] J. Escamilla, J.M. Farias, R. García, M.C. García, J.A. Sanchez, Long-term depolarization regulates the α_{1s} subunit of skeletal muscle Ca^{2+} channels and the amplitude of L-type Ca^{2+} currents, Pflugers Arch. 442 (2001) 882–890.
[33] A.R. Cantrell, V.C. Tibbs, R.E. Westenbroek, T. Scheuer, W.A. Catterall, Dopaminergic modulation of voltage-gated Na^+ current in rat hippocampal neurons requires anchoring of cAMP-dependent protein kinase, J. Neurosci. 19 (1999), RC21.