SAP97 increases Kv1.5 currents through an indirect N-terminal mechanism

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Abstract The functional interaction of the voltage-gated potassium channel hKv1.5 with the PDZ domain containing protein SAP97 has been investigated. In marked contrast with the known dependence of SAP97-induced Kv1 potassium current down-regulation on the channel C-termini, SAP97 increased hKv1.5 current through an indirect interaction with the Kv1.5 N-terminus. Deletion of the Kv1.5 N-terminus eliminated the SAP97-mediated increase in potassium currents whereas deletion of the channel's C-terminal PDZ binding motif had no effect. In contrast with other Kv1–SAP97 interactions, no physical interaction could be detected in vivo or in vitro between the two proteins. The proteins did not co-localize in cardiac myocytes nor did they co-immunoprecipitate from transfected HEK cells. Yeast two-hybrid experiments also failed to detect any interaction between the two proteins, but in one experiment of six, Kv1.5 co-immunoprecipitated very inefficiently with SAP97 from rat ventricular myocytes. Thus, we conclude that the influence of SAP97 on Kv1.5 potassium current levels is dependent upon a novel regulatory mechanism.

Key words: SAP97; PDZ; Potassium channel; Kv1.5

1. Introduction

The targeting of ion channels to their intended cellular locations is a complex process [1]. Folding and assembly occur concurrently with the synthesis of the channel proteins in the endoplasmic reticulum [2–4] and the assembled channels are then trafficked to their final destination on the cell surface or retained in intracellular pools (reviewed in [5]). This transport or internal retention of the assembled channels is influenced by signal sequences that are undoubtedly recognized by ancillary proteins whose job it is to target the channels to their programmed locations in the cell [6–8]. For example, a VXXSL motif present in the Kv1.4 C-terminus allows efficient trafficking of that channel to the cell surface whereas mutation to VXXSN (as exists in wild-type Kv1.2) yields channels that traffic poorly [6]. Pore sequences have also been implicated in channel trafficking [8]. The anchoring of the channels at their final destinations requires still other signal sequences. PSD95, for example, binds an extreme C-terminal motif present in many ion channels [9–12] and has been shown to anchor voltage-gated potassium channels at the membrane [12]. SAP97, another PDZ protein closely related to PSD95, was found by Tiffany et al. [12] to retain heterologously expressed Kv channels in the cell interior and to prevent their trafficking to the surface. Probably hundreds of PDZ proteins like PSD95 and SAP97 are expressed in human tissues. A SMART search of human genome project data [13] turns up 259 potential genes encoding PDZ domains.

Recently, we have found a second PDZ binding domain in Kv1-type potassium channels [14]. In addition to C-terminal binding motifs, we found that PSD95 binds an N-terminal sequence within or very near the T1 assembly domain. Deletion of either binding region profoundly influenced the expression of hKv1.5 in HEK293 cells expressing both the channel and PSD95. PSD95 is abundant in neurons where it localizes to the postsynaptic density and associates with a number of proteins including ion channels (reviewed in [15,16]). PSD95 is reportedly not present in the heart, however [17]. Instead, a number of related PDZ proteins are present in that tissue. One or more of these proteins might well perform a similar function in influencing Kv channel expression in that organ. Among the many possible cardiac PSD95 analogs are ZASP, ENH and SAP97 [18–20]. SAP97 is highly homologous to PSD95 and is abundant in the heart [20]. It has also been recently reported to co-localize with Kv1.5 in cardiac myocytes and to co-immunoprecipitate with the channel when the two are co-expressed in COS-7 cells [21]. Singularly among Kv1 channels, the expression of which is normally down-regulated by SAP97 [11,12], co-expression of Kv1.5 with SAP97 causes a large increase in cellular current levels [21]. We have further investigated this interaction in an attempt to learn whether this PDZ protein might also bind the N-terminus of hKv1.5 in a manner analogous to the hKv1.5–PSD95 interaction. Indeed, we found that SAP97 co-expression significantly increased Kv1.5 currents in transfected HEK cells and that the N-terminus is essential to this effect. Surprisingly, we could find no evidence of binding of the two proteins in transfected HEK293 cells or via yeast two-hybrid assays and scant evidence for any interaction in heart. Thus, SAP97 modulation of Kv1.5 currents is likely through an indirect mechanism.

2. Materials and methods

2.1. DNA constructs

For expression in HEK293 cells, SAP97 was cloned as an *Eco*RI fragment into pcDNA3 (Invitrogen, Carlsbad, CA, USA). For the yeast two-hybrid assays an *Eco*RI-*Bst*EII fragment of SAP97 contain-

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ing the three PDZ domains and half of the SH3 domain was cloned into pGAD424 and sequenced to confirm the correct reading frame. Other constructs were as previously described [14].

2.2. Electrophysiological procedures

Procedures were as previously described [14] except that HEK293 cells stably expressing Kv1.5 were transfected with pGFP and pcDNA3 or SAP97::pcDNA and pGFP.

2.3. Myocyte isolation, immunolabeling and imaging

Myocytes were isolated and prepared using the method of Scriven et al. [22]. Isolated myocytes were fixed with 2% paraformaldehyde for 10 min followed by neutralization with glycine buffer for 10 min. They were then washed with phosphate-buffered saline (PBS) for 10 min, made permeable with 1 μl/ml PBS Triton X (10 min), washed again for 10 min with PBS, and finally stored in PBS–azide solution. Cells were plated onto poly-L-lysine-coated coverslips for a minimum of 3 h at room temperature or overnight at 4°C. Myocytes were labeled for 3 h at room temperature or overnight at 4°C with primary mouse monoclonal antibodies against SAP97 (1:300, BD Transduction, San Jose, CA, USA), and rabbit polyclonal antibodies developed in our laboratory against Kv1.5 (1:300). Secondary Alexa 594-conjugated goat anti-mouse and Alexa 488-conjugated goat anti-rabbit (Molecular Probes, Eugene, OR, USA) antibodies were incubated for 2 h at room temperature. Cells were washed three times with PBS prior to mounting with a 90% glycerol, 2.5% w/v DABCO–PBS solution. Images of labeled cells were taken using a Bio-Rad Radiance Plus on an inverted Zeiss Axiovert microscope, using Bio-Rad LaserSharp 2000 software. Images were prepared using NIH Image and Photoshop Software Packages.

2.4. Co-immunoprecipitations

Co-immunoprecipitations from transfected HEK293 cell lines were as previously described [14] except that a mouse monoclonal antibody to SAP97 was employed as appropriate. In cardiac experiments, excised ventricular or atrial tissue was added to ice-cold non-denaturing lysis buffer (20 mM HEPES, 150 mM NaCl, 2 mM EDTA, 10% glycerol, 0.5% IGEPAL CA-630, 1% hemoglobin, 10 mM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride and 0.2 TIU/ml aprotinin), and underwent homogenization, two pulses of 30 s using an Ultra-Turrax T25 homogenizer (Janke and Kunkel, Staufen, Germany). The lysate was transferred to 1.5 ml Eppendorf tubes and nuclei and debris pelleted by spinning at $1000 \times g$ for 10 min, the supernatant was transferred to a fresh tube and pre-cleared twice with pre-washed CL4B Sepharose beads. The pre-cleared supernatant was mixed with 3–7 μl of antibody and incubated on ice for 1 h. Pre-swelled and washed protein A-Sepharose beads were added to the lysate/antibody mixture and incubated for 1 h with frequent mixing. The beads were pelleted and the supernatant removed. Beads were then washed three times with TSA-T (10 mM Tris pH 8.0, 140 mM NaCl, 0.1% Triton X-100), once with TSA (10 mM Tris pH 8.0, 140 mM NaCl) and once with 50 mM Tris pH 6.8. Beads were then suspended in sodium dodecyl sulfate (SDS) sample buffer and heated to 95°C for 5 min before being run on a 12% SDS–polyacrylamide gel. Proteins were then transferred to polyvinylidene difluoride membranes and probed with either rabbit anti-Kv1.5 (1:10 000; developed in our lab), mouse anti-SAP97 (1:5000) or mouse anti-actinin (1:10 000, Sigma, St. Louis, MO, USA) and horseradish peroxidase-conjugated goat anti-rabbit IgG or sheep anti-mouse (1:10 000; Jackson Laboratories). Antibody binding was detected using a chemiluminescent reagent (Renaissance, New England Nuclear).

2.5. Yeast two-hybrid experiments

Kv1.5 and Kv1.4 N- and C-terminal fragments were cloned into appropriate pGBD vectors; SAP97 and α-actinin2 were cloned in pGAD vectors [23]. For two-hybrid growth assays, yeast strain PJ69-4a was transformed with appropriate pairs of clones. Growth on media lacking adenine and histidine was monitored to assay for interaction. β-Galactosidase assays were conducted in the Y190 strain as previously described, [14].

3. Results

3.1. Co-expression of SAP97 with hKv1.5 enhances hKv1.5 currents in HEK cells

As a first step towards understanding the role of SAP97 in Kv1.5 expression, we co-expressed hKv1.5 and SAP97 in HEK cells. To accomplish this, a SAP97 construct was transfected into HEK293 cells that stably express hKv1.5. Experiments were carried out so that the electrophysiologist was blinded to the transfection group (SAP97 or vector alone). As illustrated in Fig. 1, hKv1.5 currents were significantly enhanced in the presence of the SAP97 construct 24 h after transfection. There was no apparent change in the activation or inactivation time courses of the current. It was found that peak currents were approximately doubled (from 7.9 ± 1.4 nA to 18.6 ± 2.3 nA at +60 mV, $n = 9$) 24 h after SAP97 transfection compared to control cells that were transfected with empty vector (pGFP, $n = 8$). This effect was independent of the test potential used (Fig. 1). Thus, SAP97 has a significant effect on hKv1.5 expression levels in transfected HEK cells, a result confirming in mammalian cells that obtained by Murata et al. [21] who found that SAP97 co-expression substantially enhanced Kv1.5 currents in doubly injected *Xenopus* oocytes.

These electrophysiological data demonstrate a role for SAP97 in the regulation of hKv1.5 currents at least in heterologous cell systems. The enhancement of hKv1.5 currents could be due to a direct interaction between SAP97 or to

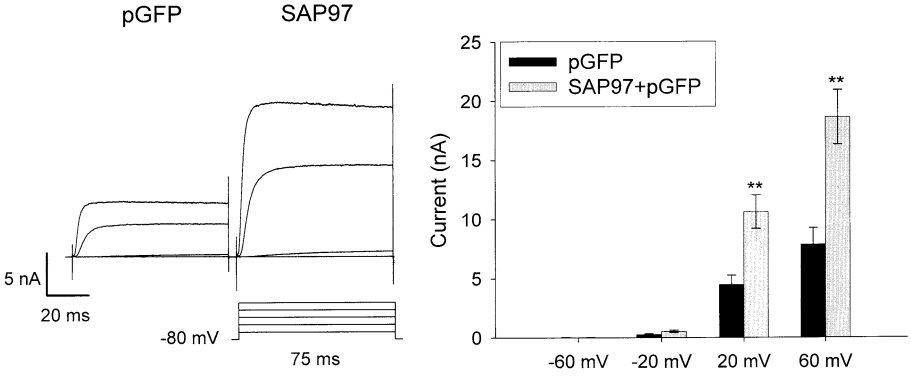

Fig. 1. Effect of SAP97 expression on hKv1.5 peak currents. HEK293 cells stably expressing hKv1.5 were used to test the effect of SAP97 co-expression on ionic currents. Cells were transiently transfected with either empty vector (pcDNA3)+pGFP or SAP97+pGFP and peak currents at several voltages were measured under voltage clamp in whole-cell configuration using a step voltage clamp protocol illustrated under the current tracings. Representative current traces at potentials of −20, −10, 20 and 60 mV are shown, and mean data ± S.E.M. (B) for eight (SAP97) or nine (pGFP) cells are presented. The asterisks indicate statistical significance with $P < 0.01$.

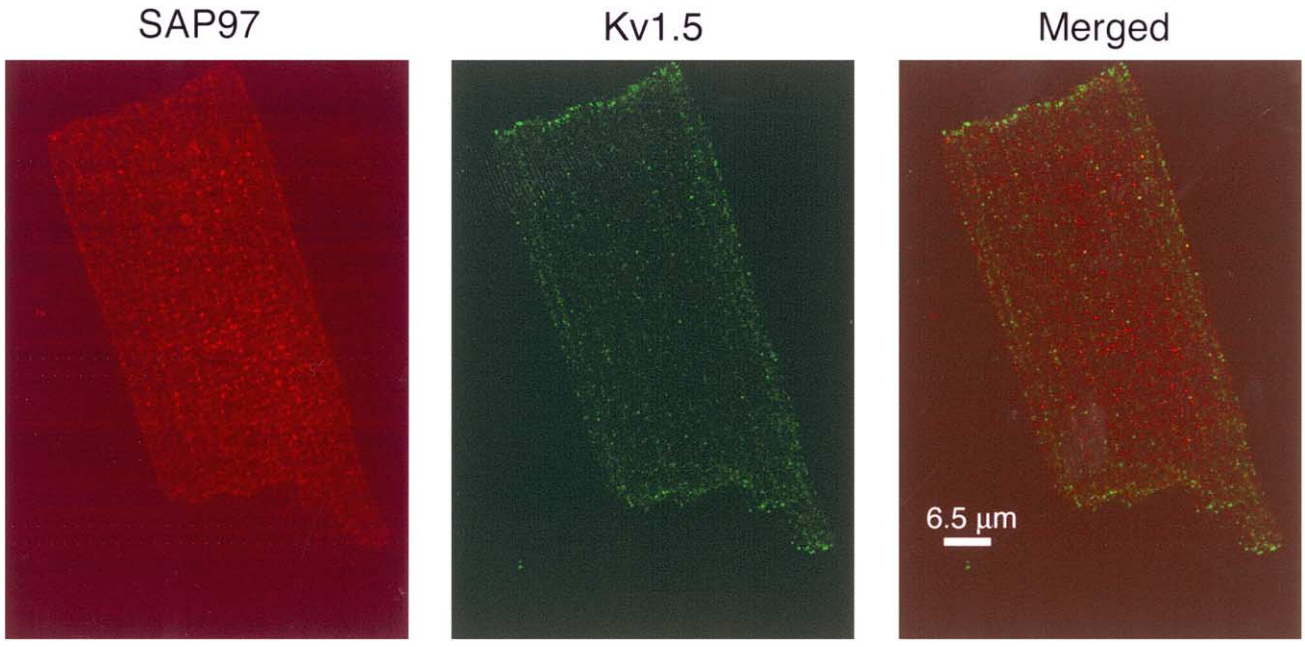

Fig. 2. SAP97 does not co-localize with Kv1.5 in rat ventricular myocytes. Confocal images of single optical slices of ventricular myocytes. SAP97 was detected with Alexa594-labeled secondary antibody (red); Kv1.5 was detected with Alexa488-labeled secondary antibody (green). Yellow indicates co-localization of the channel with SAP97 in the merged image.

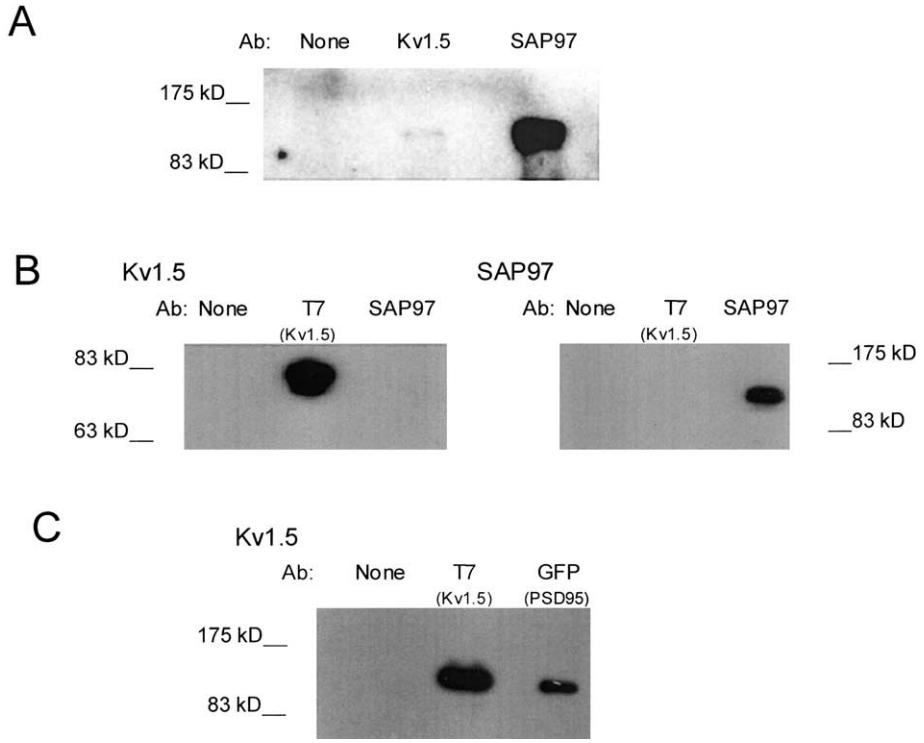

Fig. 3. Attempted co-immunoprecipitation of SAP97 and Kv1.5. A: The single example (one of six) of co-immunoprecipitation of SAP97 with Kv1.5 from rat ventricular extracts. Extracts from ventricular tissue were mixed with no antibody, anti-Kv1.5 or anti-SAP97. The protein–antibody complexes were precipitated with protein A-Sepharose and subjected to Western analysis probed with anti-SAP97. B: Kv1.5 and SAP97 fail to co-immunoprecipitate from transfected HEK293 cells. Extracts of SAP97, T7-tagged Kv1.5 double-expressing HEK cells were mixed with no antibody, anti-T7 or anti-SAP97. The complexes were precipitated with protein A-Sepharose and subjected to Western analysis probing with anti-T7 (left panel) or anti-SAP97 (right panel). C: PSD95 co-immunoprecipitates with Kv1.5 from transfected HEK cells. Control experiments were conducted as in B except that the HEK cells co-expressed T7-tagged Kv1.5 and GFP-tagged PSD95. PSD95 was immunoprecipitated with anti-GFP. The blot was probed with anti-T7.

an indirect action, either through a bridging molecule or through an effect on another molecule or molecules that somehow regulates hKv1.5 activity. To begin to distinguish between these possibilities, we conducted co-localization, co-immunoprecipitation and yeast two-hybrid experiments.

3.2. hKv1.5 and SAP97 fail to co-localize in rat cardiac myocytes

Co-localization experiments were performed to test whether hKv1.5 and SAP97 interact in vivo. Confocal images were obtained of isolated rat ventricular myocytes cross-reacted with antibodies to hKv1.5 and SAP97. As shown in Fig. 2, both Kv1.5 and SAP97 were widely distributed in the myocytes. The two molecules co-localized very poorly – if at all – in these cells, however. Kv1.5 was enriched at the intercalated disks and SAP97 was generally internal. SAP97 appeared to be largely distributed along Z-lines

3.3. Kv1.5 co-immunoprecipitations with SAP97 from cardiac myocytes

In order to further test whether or not Kv1.5 and SAP97 directly interact in atrial and/or ventricular myocytes, we conducted co-immunoprecipitation experiments. We found that while Kv1.5 and SAP97 were efficiently immunoprecipitated by their respective antibodies, SAP97 failed to co-immunoprecipitate with Kv1.5 in five of six experiments from ventricular extracts, although in one experiment out of six, SAP97 did co-immunoprecipitate very poorly with hKv1.5 (Fig. 3A). Whether this represents a real interaction or is a one-time artifact is unclear. We could not detect any co-immunoprecipitation of Kv1.5 by antibody to SAP97, nor of SAP97 with Kv1.5 from atrial extracts (data not shown). These results are consistent with the lack of co-localization seen in imaging experiments.

3.4. hKv1.5 fails also to co-immunoprecipitate with SAP97 from transfected HEK cells

While Murata et al. [21] were unable to consistently co-immunoprecipitate the proteins from the cardiac myocytes, they were able to co-immunoprecipitate SAP97 and Kv1.5 from transfected COS-7 cells. We therefore decided to test whether the two proteins could be co-immunoprecipitated from a highly over-expressed system using transfected HEK cells. HEK293 cells stably expressing a T7-tagged hKv1.5 construct were transfected with a SAP97 clone in pcDNA3 and co-immunoprecipitation experiments were conducted. As shown in Fig. 3B, both hKv1.5 and SAP97 could be readily immunoprecipitated from these cells. But antibody to one never brought down the other. This is unlikely to be due to detergent disruption of the Kv1.5–SAP97 interaction. When the closely related PDZ protein PSD95 was co-expressed in place of SAP97, PSD95 readily co-immunoprecipitated with hKv1.5 (Fig. 3C). Given that the influence of SAP97 on hKv1.5 currents in this cell line is marked, this failure of the two proteins to bind each other was quite surprising.

3.5. SAP97 interacts with Kv1.4 but not with hKv1.5 in yeast two-hybrid experiments

In a final attempt to determine whether we could detect an interaction under quite different conditions, we conducted yeast two-hybrid studies. Yeast strain PJ69-4a [23] was co-transformed with C- and N-terminal fragments of hKv1.5 or

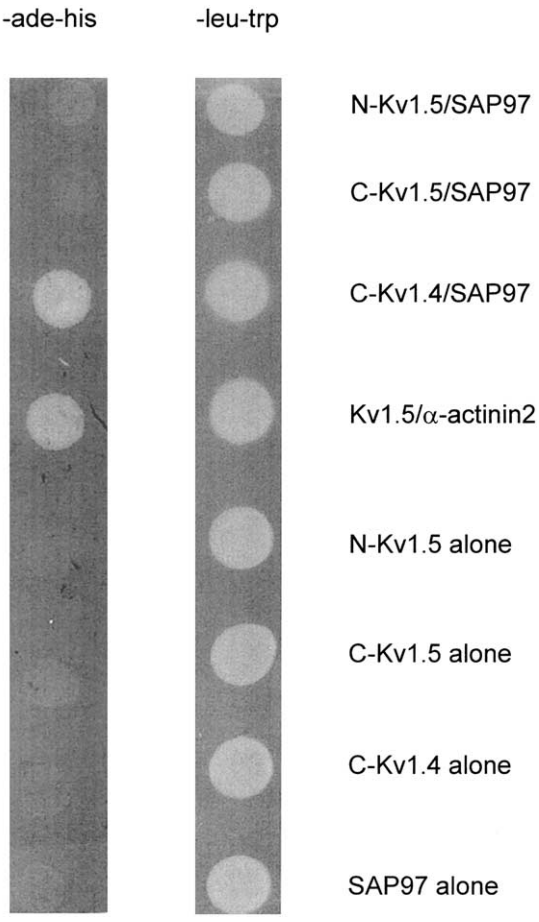

Fig. 4. Yeast two-hybrid assay for the interaction of Kv1.5 N- and C-terminal interactions with SAP97. The Kv1 channel fragments were expressed as fusions to the DNA binding domain of the yeast GAL4 protein. SAP97 and α-actinin2 clones were expressed as fusions to the GAL4 transcription activating domain. Growth on −ade, −his medium indicates a detectable interaction. Growth on −leu, −trp confirms that both fusion vectors are present in the yeast. 'Alone' refers to yeast co-transformed with the reported construct plus its empty vector partner for the yeast two-hybrid assay.

the C-terminus of Kv1.4 in pGBD and the PDZ domains of SAP97 in pGAD. As shown in Fig. 4, yeast harboring both the Kv1.4 C-terminus and the SAP97 construct grew well on medium lacking adenine and histidine as did another control in which the hKv1.5 N-terminus was tested against α-actinin2. Neither the hKv1.5 C- nor N-terminal fragments allowed growth on the test media. Similar results were obtained when the constructs were co-transformed into yeast strain Y190 and β-galactosidase assays were conducted. Only the Kv1.4 C-terminus/SAP97 and hKv1.5 N-terminus/α-actinin2 combinations yielded β-galactosidase activities above control (data not shown). While it remains formally possible that SAP97 directly interacts with an untested fragment of Kv1.5 or with a combination of fragments, these yeast two-hybrid data are inconsistent with a stereotypical interaction between the extreme C-terminus of the channel with the PDZ protein.

3.6. SAP97 enhancement of hKv1.5 currents depends on an intact Kv1.5 N-terminus

The results of all of the experiments described above are

inconsistent with a typical PDZ–extreme C-terminal interaction between SAP97 and Kv1.5. Instead, SAP97 must exert its effect by some different, most likely indirect, mechanism. To gain further insight into this issue, experiments with hKv1.5 C- and N-terminal deletion mutants were conducted. SAP97 was transfected into HEK293 cells stably expressing either hKv1.5ΔETDL or hKv1.5ΔN209 [24] and potassium currents were measured as before. The hKv1.5ΔETDL mutant lacks the C-terminal four amino acids required for interaction with PDZ proteins and hKv1.5ΔN209 lacks the N-terminal 209 amino acids of the channel. Strikingly, it was the N-terminus and not the C-terminus that proved essential to the SAP97 enhancement of Kv1.5 potassium currents. hKv1.5ΔN209 K^+ currents were not affected at all by SAP97 co-expression (Fig. 5A) but, as with the wild-type channel, SAP97 co-expression significantly increased potassium currents in the ΔETDL line (Fig. 5B). Again, this effect of SAP97 on the channel was potential-independent. HEK cells display a small endogenous potassium current. Conceivably, the channel subunits that carry this current could heteromultimerize with the transfected Kv1.5 subunits and mediate a SAP97 interaction with those channels. To test this possibility, similar experiments in CHO and LM6 cells, which express no endogenous potassium current, were conducted. As in HEK cells, hKv1.5ΔETDL currents were approximately doubled when SAP97 was co-expressed in these cells. Peak currents at +60 mV in CHO cells expressing hKv1.5ΔETDL were 1.04 ± 0.12 nA and 2.42 ± 0.17 nA when SAP97 was co-expressed. In LM cells the corresponding currents were 2.06 ± 0.30 nA and 3.87 ± 0.66 for cells expressing only hKv1.5ΔETDL and hKv1.5ΔETDL plus SAP97, respectively. Thus, it was confirmed that the SAP97-mediated increase in Kv1.5 currents was independent of the PDZ binding motif at the channel C-terminus. This lack of dependence of the SAP97 effect on the C-terminal acids of Kv1.5 is in stark contrast to the results with other Kv1 channels [11,12]. A novel regulatory mechanism must underlie the SAP97-mediated increase in Kv1.5 potassium currents.

3.7. α-Actinin2 co-immunoprecipitates with both hKv1.5 and SAP97

The pattern of SAP97 staining in the confocal imaging experiments (Fig. 2) suggests that the protein might be in large measure localized to T-tubules or to Z-disks. Occasionally, a

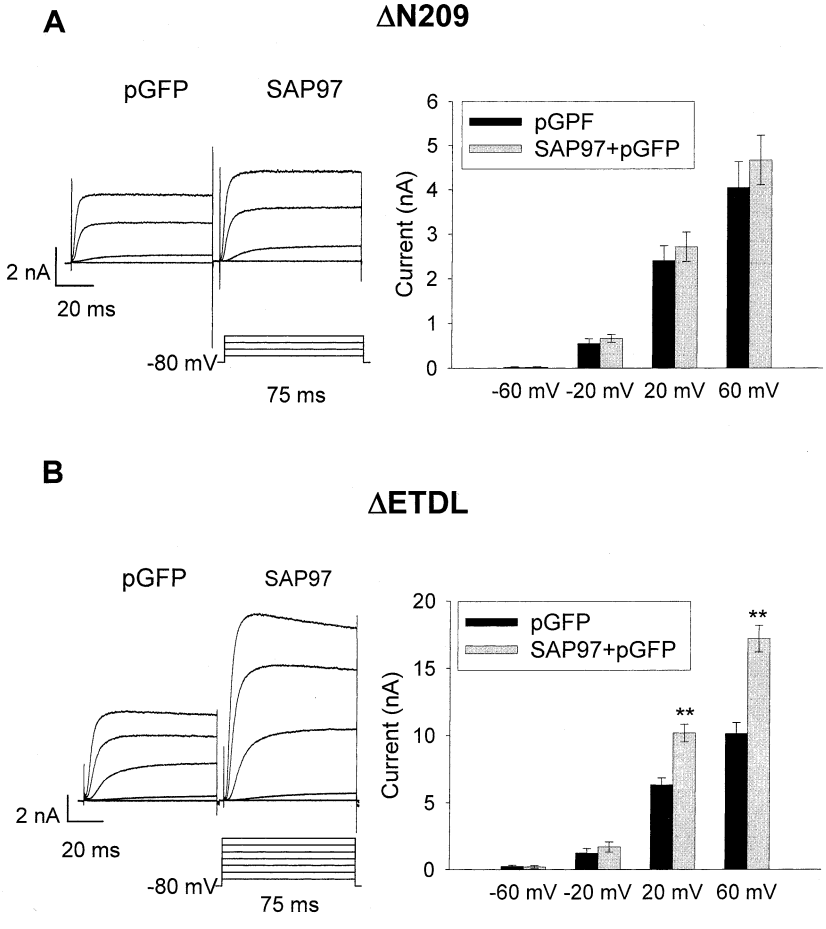

Fig. 5. A: SAP97 fails to increase Kv1.5ΔN209 peak currents. HEK293 cells stably expressing Kv1.5ΔN209 were used to test the effect of SAP97 co-expression on peak currents. Representative current traces are shown on the left. The bar graph illustrates the mean currents ± S.E.M. for four (SAP97) and seven (pGFP) cells. B: SAP97 increases Kv1.5ΔETDL peak currents. HEK293 cells stably expressing Kv1.5ΔETDL were used to test the effect of SAP97 co-expression on peak currents. Representative current traces (A) and mean data ± S.E.M. (B) for nine (SAP97) and nine (pGFP) cells are presented. In both A and B, cells were transiently transfected with either empty vector (pcDNA3)+pGFP or SAP97+pGFP and peak currents at several voltages were measured under voltage clamp in whole-cell configuration. The asterisks indicate statistical significance at $P < 0.01$.

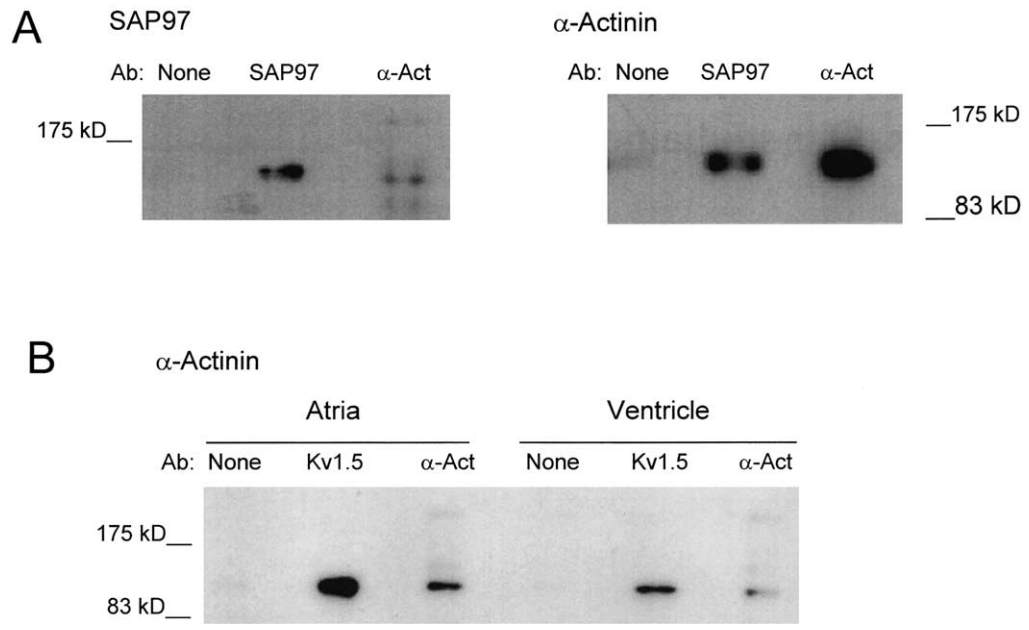

Fig. 6. Both Kv1.5 and SAP97 interact with α-actinin2 in rat heart. A: Co-immunoprecipitation of SAP97 with α-actinin2 from rat ventricular extracts. Extracts from ventricular tissue were mixed with no antibody, anti-α-actinin2 or anti-SAP97. The protein–antibody complexes were precipitated with protein A-Sepharose and subjected to Western analysis probed with anti-SAP97 (left panel) or anti-α-actinin2 (right panel). B: Kv1.5 and α-actinin2 co-immunoprecipitate from rat cardiac extracts. Extracts from atrial or ventricular tissue were mixed with no antibody, anti-α-actinin2 or anti-Kv1.5. The protein–antibody complexes were precipitated with protein A-Sepharose and subjected to Western analysis. Blots were probed with anti-α-actinin2.

similar but weaker Z-band-type staining pattern can be seen in Kv1.5 imaging experiments as well (data not shown). One of the major proteins at the Z-line is α-actinin2 [25], a protein we have previously demonstrated to interact with hKv1.5 in heterologous cells [24]. To test whether there is any potential for a role for actinin in the SAP97–Kv1.5 interplay, co-immunoprecipitation of Kv1.5 with α-actinin2, and of SAP97 with α-actinin2 was attempted from cardiac myocyte lysates. As shown in Fig. 6, both SAP97 (Fig. 6A) and Kv1.5 (Fig. 6B) co-immunoprecipitated well with α-actinin2. While actinin may or may not be involved in the SAP97 enhancement of hKv1.5 currents, it could well underlie the weak co-immunoprecipitation of SAP97 with Kv1.5 in cardiac cells. Limited co-association of the two proteins with Z-band α-actinin2, for example, could be more than sufficient to yield the results shown in Fig. 3A.

4. Discussion

We have recently shown that PSD95, a protein closely related to SAP97, binds both the C- and N-termini of hKv1.5 via its PDZ domains [14]. PSD95 is reportedly not expressed in heart [17] and we wondered whether the N-terminal PDZ binding domain in Kv1.5 had any relevance to the channel's regulation in that organ. For this reason, we examined the interaction between Kv1.5 and SAP97, a PDZ protein that is widely expressed in mammalian heart [20].

Without a doubt, SAP97 significantly increases hKv1.5 currents in heterologous cells. In this regard, our results are similar to those reported by Murata et al. [21] using *Xenopus* oocytes. At first glance, it seems surprising, therefore, that we could find very little evidence for any direct interaction between the two proteins. But the effect of SAP97 co-expression on Kv1.5 currents is exceptional and dramatically different from its known effects on other Kv1 channels. Kv1.1, Kv1.2, Kv1.3 and Kv1.4 channels are all clustered internally by SAP97 and their surface expression is substantially reduced [11,12]. Indeed, given this stark difference in effects of SAP97 on most Kv1 channels versus its effect on Kv1.5, it would be surprising if SAP97 did in fact bind Kv1.5. While Murata et al. report that the two proteins do interact, they, like us, had difficulty detecting these interactions in the heart. They were unable to co-immunoprecipitate the two proteins from that tissue and, importantly, their co-localization experiments employed a fairly non-specific antibody directed against PSD95. The most likely explanation for the co-localization they report in myocytes is that they detected an overlap between Kv1.5 and a PDZ protein other than SAP97. The SAP97 distribution they report in their myocytes is unusual in that it is concentrated at the cell surface and at the intercalated disk. In our hands, and those of Leonoudakis et al. [20], cardiac myocyte SAP97 staining was mainly intracellular along the Z-disks or perhaps at T-tubules. Kv1.5, on the other hand, was most prevalent at the intercalated disks.

How can SAP97 affect Kv1.5 currents yet have no physical interaction with the channel? It remains formally possible that Kv1.5 and SAP97 do directly interact but that the interaction is fleeting. We cannot exclude the possibility that phosphorylation or some other modification(s) of the channel or SAP97 influences binding. Phosphorylation at a C-terminal serine residue is known to disrupt the interaction of PSD95 with Kir2.3 [26]. Similar phosphorylation inhibits α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate receptor subunit glucose receptor-2 binding to the PDZ domains of GRIP but not of PICK1 [27,28]. Conceivably differences in intrinsic modification rates in various cell types could explain the markedly different re-

sults we obtained in co-immunoprecipitations from transfected HEK cells and those obtained by Murata et al. in COS-7 cells. Perhaps in COS-7 cells the state of the channel is permissive of a stable SAP97-Kv1.5 interaction that is not seen in other cell types (including heart).

But even if SAP97 does bind the Kv1.5 C-terminus under specific conditions, that fact cannot explain the lack of dependence of the SAP97-mediated current enhancement on the extreme Kv1.5 C-terminus. Indeed, it would appear that the Kv1.5 N-terminus, or at least some part of the channel affected by the N-terminus, not the C-terminus, is necessary for the SAP97 effect. This must be mediated by a different mechanism than is dominant with the other Kv1 channels. One possibility is that SAP97 frees Kv1.5 to traffic to the surface by titrating away another binding protein(s). Similarly, it could free another Kv1.5 binding protein to traffic to the surface, and then stabilize the channel there. SAP97 might even affect Kv1.5 processing or its transcript levels by an as yet unidentified cascade. Any of these scenarios would be consistent with our near-complete failure to detect any molecular interaction Kv1.5 and SAP97.

We found that both Kv1.5 and SAP97 interact strongly with α-actinin2. Thus, it is conceivable that actinin could be involved in the SAP97 enhancement of Kv1.5 currents. For example, α-actinin2 might serve as a bridge between the two molecules. Indeed, SAP97 cardiac myocyte staining suggests that SAP97 localizes to the Z-bands, structures very rich in α-actinin2 [29], and, in occasional cells, some Kv1.5 appears similarly distributed. It is hard to reconcile this actinin bridge hypothesis with the failure of SAP97 and Kv1.5 to co-localize in the majority of myocytes, however. Actinin is too small a molecule for its length to account for a complete lack of Kv1.5–SAP97 overlap. If actinin is involved, perhaps it is more likely that it is titrated away from Kv1.5 by SAP97. If, for example, actinin binding to Kv1.5 anchors the channel in the cell interior, SAP97 binding to an overlapping site in actinin could free the channel to traffic to the cell surface. Such a possibility would be consistent, at least, with the known effects of disruption of the cytoskeleton and α-actinin2 antisense experiments. Kv1.5 currents are greatly increased by these treatments [24].

In summary, we have found that SAP97 has a profound influence on Kv1.5 currents in heterologous cells. This effect is dependent on an intact Kv1.5 N-terminus, yet little evidence for a direct interaction between the two proteins could be found. Further research will be required to determine the exact mechanism by which SAP97 increases Kv1.5 K^+ currents.

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