

# Expression, regulation and role of the MAGUK protein SAP-97 in human atrial myocardium<sup>☆</sup>

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## Abstract

**Objective:** In various cell types, membrane-associated guanylate kinases proteins called MAGUK play a major role in the spatial localization and clustering of ion channels. Here, we studied the expression and role of these anchoring proteins in human right atrial myocardium by means of various molecular, biochemical and physiological methods. **Methods and results:** SAP-97, PSD-95, Chapsyn and SAP-102 messengers were detected by reverse transcriptase–polymerase chain reaction (RT-PCR) on mRNA extracted from both whole myocardium and isolated myocytes. Western blot revealed that the MAGUK protein SAP-97 and, to a lesser extent, PSD-95, is abundantly expressed in human atrial myocardium, while Chapsyn are almost undetectable. Confocal microscopic visualization of cryosection of atrial myocardium stained with the anti-PSD-95 family antibody showed positive staining at the plasma membrane level and cell extremity. Calpain-I cleaved both SAP-97 and PSD-95 proteins, resulting in an accumulation of short bands, including an 80-kDa band that was also detected in the cytosolic protein fraction. Immunoprecipitation of SAP-97 co-precipitated hKv1.5 channels, and vice versa. Co-expression of cloned SAP-97 and hKv1.5 channels in Chinese hamster ovarian (CHO) cells increased the K<sup>+</sup> current ( $157.00 \pm 19.45$  pA/pF vs.  $344.50 \pm 58.58$  pA/pF at +50 mV). **Conclusions:** The protein SAP-97 is abundantly expressed in human atrial myocardium in association with hKv1.5 channels, and probably contributes to regulating the functional expression of the latter.

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**Keywords:** Arrhythmia; Ion channels; K-channel

## 1. Introduction

A family of anchoring proteins named MAGUK (for Membrane Associated GUanylate Kinase) has emerged as a key element in the organization of protein complexes in specialized membrane regions. These proteins are characterized by the presence of three protein–protein interaction PDZ domains (for Postsynaptic, Disc large, Zonula occludens), an SH3 domain, and a guanylate kinase-like

domain [1–5]. The MAGUK family comprises the post-synaptic density-95 (PSD-95) protein and closely related molecules like Chapsyn-110, SAP-102 and SAP-97 [6]. These are located either on the pre- and/or post-synaptic sides of synapses or at cell–cell adhesion sites of epithelial cells [7,8]. MAGUK proteins interact with glutamate receptors [3] pumps such as the plasma membrane Ca<sup>2+</sup>-ATPase [9] and various ionic channels [2,10]. For instance, an interaction has been reported between MAGUK and several channels such as Kv and Kir, via an S/T-X-V/L sequence usually located at C-terminal extremity of the channel and the first two PDZ domains of MAGUK proteins [10–13]. The role of these anchoring proteins in

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channel function is not fully understood. When co-expressed with cloned channels in cell lines, MAGUK proteins usually enhance the current density by increasing the number of functional channels to the sarcolemma [2,10,12–14]. In addition, these anchoring proteins can facilitate signaling between channels and several enzymes or G protein-dependent signaling pathways [15].

In heart, the role of MAGUK proteins in regulating the membrane expression and clustering of ionic channels are still poorly known. Recent studies report that the MAGUK protein SAP-97 is expressed in rat ventricular myocytes co-localized with potassium Kir2.2 [16] and Kv1.5 channels [12] at the level of the intercalated disc and t-tubule system. Moreover, Murata et al. [12], demonstrate that the protein SAP-97 interacts with the *Shaker* channel Kv1.5 expressed in cell line and increases the Kv1.5 current. In human atrial myocardium, hKv1.5 *shaker* channels that carries a large component of the outward current [17–19] are concentrated in the intercalated disc where they can interact with various proteins [20]. This suggests that in this tissue also, scaffolding MAGUK proteins could regulate the subcellular localization of hKv1.5 channels.

The present study was undertaken to identify the MAGUK proteins expressed in human atrial myocardium and to determine their role in regulating the functional expression of potassium channels in this tissue. We present evidence that MAGUK proteins, and notably SAP-97, are abundant in human atrial myocardium and that they interact with endogenous hKv1.5 *shaker* channels. Preliminary results of this study were presented at 71th session of the American Heart Association [21].

## 2. Methods

### 2.1. Tissue samples and myocyte isolation

With approval from the ethics committee of our institution, biopsy specimens of right atrial appendage were obtained from 34 adult patients aged from 29 to 83 years undergoing heart surgery for coronary artery disease ( $n=20$ ), mitral valve disease ( $n=6$ ), aortic valve disease ( $n=8$ ). Samples were frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  as previously described [22]. Adult rat brains were frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ .

### 2.2. RT-PCR

Total RNA was extracted from tissue or isolated myocytes using the phenol chloroform method [23] or Trizol<sup>®</sup> (Life technologies) procedures, respectively, and were then reverse-transcribed using M-MLV reverse transcriptase (Life technologies) and Oligo-dt according to the manufacturer's guidelines. Reverse-transcribed (RT) RNA (100 ng) was submitted to polymerase chain reaction amplification (PCR) in a 25- $\mu\text{l}$  reaction mixture (annealing

temperature  $55^{\circ}\text{C}$ , 30 cycles). The following primers were used: (for PSD-95, accession number: U83192) 5'-AG-GTG-GCA-GAG-CAG-GGG-AAG-3' (sense), 5'-AGGGCTTGGGCAATTCAGTC-3' (antisense); (for SAP-97, accession number: U13897) 5'-AA-GTA-GCA-GGA-AAG-GGC-AAA-CAC-TG-3' (sense), 5'-CCAGAG-GAAAGGGCAAAGAGATG-3' (antisense); (for Chapsyn-110, accession number: U32376) 5'-AG-GTG-GAT-GGC-AGA-GAC-TAT-CAC3' (sense), 5'-CTAAATGCTCTTCTGTCGTTGTCAG3' (antisense); (for SAP-102, accession number: U49089) 5'-AA-ATG-GAG-AAA-GAT-ATT-CAG-GAC-AAC-3' (sense), 5'-GGAGGAGGGAAGAGGGACTTG-3' (antisense) and (for ZO-1, accession number: L14837) 5'-CGA-GGC-ATA-TTT-AAC-AGC-AAT-GG-3' (sense), 5'-CAACAGTCCCGTCAATCACAAAC-3' (antisense). All PCR primers were chosen so that the sense primer was within the coding sequence close to the stop codon and the reverse primer was within the 3' untranslated region (UTR), in order not to discard any possible spliced isoforms.

### 2.3. Protein extraction

Atrial myocardium and rat brain tissue were ground with a mortar and pestle in liquid nitrogen and homogenized on ice with a glass/Teflon homogenizer in 10 volumes of Tris buffer (in mM) 10, EDTA 5, pH 7.4 in the presence of the following enzyme inhibitors: iodoacetamine 1 mM, AEBSF 0.5 mM, aprotinin 10  $\mu\text{g}/\text{ml}$ , leupeptin 10  $\mu\text{g}/\text{ml}$ , pepstatin 1  $\mu\text{g}/\text{ml}$ , and  $\text{Na}_3\text{VO}_4$  1 mM. The homogenates were spun at  $800\times g$  for 10 min at  $4^{\circ}\text{C}$  to pellet the nuclei and debris, then again at  $10\,000\times g$  for 10 min at  $4^{\circ}\text{C}$ ; finally, the supernatants were centrifuged at  $105\,000\times g$  for 1 h at  $4^{\circ}\text{C}$  (Beckman TL-100 Ultracentrifuge). The resulting supernatant (cytosolic fraction, C) was saved and membrane pellets were resuspended in extraction solution supplemented with 2% Triton X-100 (membrane triton, MT); both fractions were stored at  $-80^{\circ}\text{C}$ . In some experiments, before ultracentrifugation, the lysates were incubated with 0.6 M KI to dissociate actin from membrane constituents.

For protein phosphorylation studies,  $\text{Na}_3\text{VO}_4$  was omitted from the extraction buffer and dephosphorylation was performed by adding 200 IU of calf intestinal alkaline phosphatase (CIAP) (Gibco) to 30  $\mu\text{g}$  of MT with incubation at 37 and  $50^{\circ}\text{C}$  in a water bath for 1 h.

For calpain experiments, protein was extracted using a Tris-acetate buffer (100 mM, pH 7.4) but without enzyme inhibitors; a final protease concentration of between 0.15 and 3.6 U/ml was used for 30  $\mu\text{g}$  of membrane protein. To obtain a free  $\text{Ca}^{2+}$  concentration of 1  $\mu\text{M}$ , 4 mM  $\text{CaCl}_2$  and 10 mM EGTA were added to the solution. Protein was incubated in this solution for 30 min at  $37^{\circ}\text{C}$ . The calpain type I and II inhibitors leupeptin and iodoacetamide were

used to inhibit protease activity. All reagents were purchased from Sigma (St. Louis, MO).

#### 2.4. Immunoblotting and immunoprecipitation

Then, 30 µg of protein was solubilized in 5× reducing sample buffer, fractionated on 8% polyacrylamide–sodium dodecyl sulfate (SDS) gels and transferred to PVDF membrane (NEN). Blots were then blocked with PBST containing 5% defatted milk and incubated overnight in PBST containing 0.5% defatted milk and the following primary antibodies: mouse monoclonal anti-PSD-95 clone k28/43 (Upstate Biotechnology), mouse monoclonal anti-PSD-95-family for pdz<sub>1-2</sub> domains (1/2000, Upstate Biotechnology), rabbit polyclonal anti-Chapsyn-110 (1/500, Alomone labs), or rabbit polyclonal anti-rat Kv1.5 α-subunit (1/500, Alomone, anti-Kv1.5-A). We checked that the anti-Kv1.5-A recognized the cloned human Kv1.5 and that the reaction was inhibited by preincubation of the antibody with the immunizing peptide. Blots were then washed three times, incubated with peroxidase-conjugated secondary antibodies (AffinityPure goat anti-rabbit IgG and goat anti-mouse IgG, Jackson Immunoresearch) (1/10 000) for 1 h, washed three times with phosphate buffer saline, then incubated with the detection reagent, ECL (NEN) and autoradiographed.

Goat anti-mouse IgG M-450 and sheep anti-rabbit IgG M-280 coated Dynabeads (Dyna) were used for immunoprecipitation. Briefly, 4 µg of anti-PSD-95-family or 4 µg of anti-Kv1.5-A or anti-Kv1.5 α-subunit clone K7/45 (anti-Kv1.5-B; Upstate laboratories) were incubated overnight with 10<sup>8</sup> beads in 250 µl of PBS with gentle rotation. Then MT or C fractions were added to the appropriate beads in 250 µl of PBS and rotated overnight at 4 °C. After magnetic sorting, the beads were washed three times with PBS, re-suspended in 200 µl of sample buffer and boiled for 10 min; immunoprecipitated proteins were recovered by centrifugation.

#### 2.5. Immunohistochemistry

Indirect immunofluorescence was performed on human right atria frozen sections (6 µm) as previously described [22]. Briefly, sections were fixed with methanol (10 min at –20 °C) for PSD-95 family protein, washed in phosphate-buffered saline (PBS) containing 5% BSA to block non specific binding sites then incubated overnight at 4 °C with mouse anti-PDZ-95 family antibodies (Upstate biotechnology) and revealed with horse biotinylated antimouse IgG secondary antibodies (Vector Laboratories, Abcys) then streptavidin-Texas red (Amersham). For hKv1.5 channel immunostaining, unfixed section were blocked with 5% BSA in PBS containing 0.1% Triton X-100, then incubated overnight at 4 °C with rabbit anti-mouse Kv1.5 antibodies (Alomone Labs) and revealed with Alexa Fluor<sup>®</sup> 488 goat anti-rabbit IgG secondary antibodies (Molecular Probes).

In control experiments the primary antibodies were omitted. Slides were examined with a Zeiss LSM-510 confocal scanning laser microscope equipped with a 25-mW argon laser and a x63 (oil) objective with numerical aperture of 1.4. Green fluorescence was observed with a 505–550 nm band-pass emission filter under 488 nm laser illumination and red fluorescence was observed with a 560 nm long-pass emission filter under 543 nm laser illumination. The resulting images were printed on an Epson Stylus Photo.

#### 2.6. Cell preparation and transfection

Chinese hamster ovarian cells (CHO) were cultured in HAM F12 medium (Gibco Nutrient Mixture) supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin B (Gibco BRL). hKv1.5 cDNA was generated by PCR and checked by sequencing. Rat SAP-97 cDNA was a generous gift from Dr Hata (Tokyo Medical and Dental university, Tokyo, Japan) and Dr Takai (Osaka University Graduate School of Medicine, Osaka, Japan) [10]. The hKv1.5 and SAP-97 cDNA were inserted into the expression vector pIRES2-EGFP (Clontech) and into our home-made vector pIRES2-DsRed, respectively, to check for co-expression of the SAP-97 and hKv1.5 constructs. Transfection was performed using the FuGENE-6 transfection reagent (Roche Molecular Biochemicals) as previously described [24]. Two days before current recordings with 0.5 µg of Kv1.5 cDNA and 1.5 µg of SAP-97 cDNA or 1.5 µg DsRed cDNA (to study hKv1.5 alone). Between 90 and 100% of green and red fluorescent cells had a current.

#### 2.7. Current measurements

Whole-cell patch-clamp currents were recorded with borosilicate glass pipettes (resistance 1.5–2 MΩ) connected to the input stage of a patch-clamp amplifier (Axoclamp 200A, Axon Instrument). Resistance in series was compensated to obtain the fastest capacity transient current; but not the capacitive and leakage currents. Currents were filtered at 5 kHz, digitized with a Labmaster (Lab Rac, Scientific Solution) and stored on the hard disk of a personal computer. Data were acquired and analyzed with Acquis-1 software (G. Sadoc, CNRS, Gif/Yvette, France).

The conductance  $G$  was calculated with equation:  $G = I / (V - E_k)$ ;  $E_k$  is the equilibrium potential for K<sup>+</sup> calculated with the Nernst equation (–85 mV in our experimental conditions);  $I$  is the current measured at the end of the test pulse for each membrane potential ( $V$ ). Data on the conductance/voltage activation curve were best fitted with a Boltzmann distribution equation:  $G/G_{\max} = 1/[1 + \exp((V_{1/2} - V)/k)]$ , where  $G$  represents the conductance calculated at membrane potential  $V$ ,  $V_{1/2}$  the potential at which half of  $G_{\max}$  was obtained, and  $k$  the slope factor. The  $V_{1/2}$  and  $k$  values were calculated for each individual

experiment and thereafter, the mean value for  $V_{1/2}$  and  $k$  values for each group of experiments was calculated.

## 2.8. Solutions and drugs

Cells were bathed in an external solution containing (in mM) NaCl 137, KCl 5.4,  $\text{CaCl}_2$  2,  $\text{MgCl}_2$  1, HEPES 10 and glucose 10, adjusted to pH 7.3 with NaOH. Patch pipettes were filled with an internal solution containing (in mM): K-aspartate 115, KCl 5, MgATP 5, Na-pyruvate 5,  $\text{MgCl}_2$  3, EGTA 4, and HEPES 10, adjusted to pH 7.2 with KOH [24]. 4-aminopyridine (4-AP) was dissolved in the extracellular solution; In some experiments, NaCl in the external solution was replaced with an equimolar concentration of tetraethylammonium (TEA). All experiments were carried out at room temperature.

## 2.9. Statistical analysis

Data are presented as means  $\pm$  S.E.M. Student's unpaired *t*-test, as appropriate, was used to determine the significance of differences. *P* values of  $<0.05$  were considered significant.

## 3. Results

### 3.1. Several MAGUK proteins are expressed in human atrial myocardium

As illustrated in Fig. 1, SAP-97, PSD-95, Chapsyn, SAP-102 and ZO-1 mRNA was detected in both human atrial myocardium (Fig. 1A) and isolated myocytes (Fig. 1B). We then studied the expression of MAGUK proteins known to interact with ionic channels in various cell types, by means of western blot and immunocytochemistry. As a positive control, we used membrane protein extracted from rat brain tissue, that is known to contain a number of MAGUK proteins, including SAP-97, that migrates as a doublet (around 140 kDa), Chapsyn (around 110 kDa), PSD-95 (around 95 kDa) and also a lower protein at around 80 kDa (Fig. 2). With human atrial myocardium membrane protein, the anti-PSD-95 family antibody strongly detected the doublet at 140 kDa (SAP-97 protein), together with a weaker band at 95 kDa (also seen with the anti-PSD-95 antibody), and faint bands around 110 kDa (also weakly visualized with the anti-Chapsyn antibody). Both anti-PSD-95-family and PSD-95-specific antibodies cross-reacted with the 80 kDa protein (Fig. 2). This pattern of expression of the MAGUK proteins was observed in all specimen studied and whatever the clinical history of the patients. Staining of cryosections of atrial myocardium with anti-PSD-95 family antibody yielded a staining at the level of the intercalated disk (Fig. 3A, arrowhead) and at the periphery of myocytes (Fig. 3A, arrow). As previously reported [20], the hKv1.5 channel was also predominantly

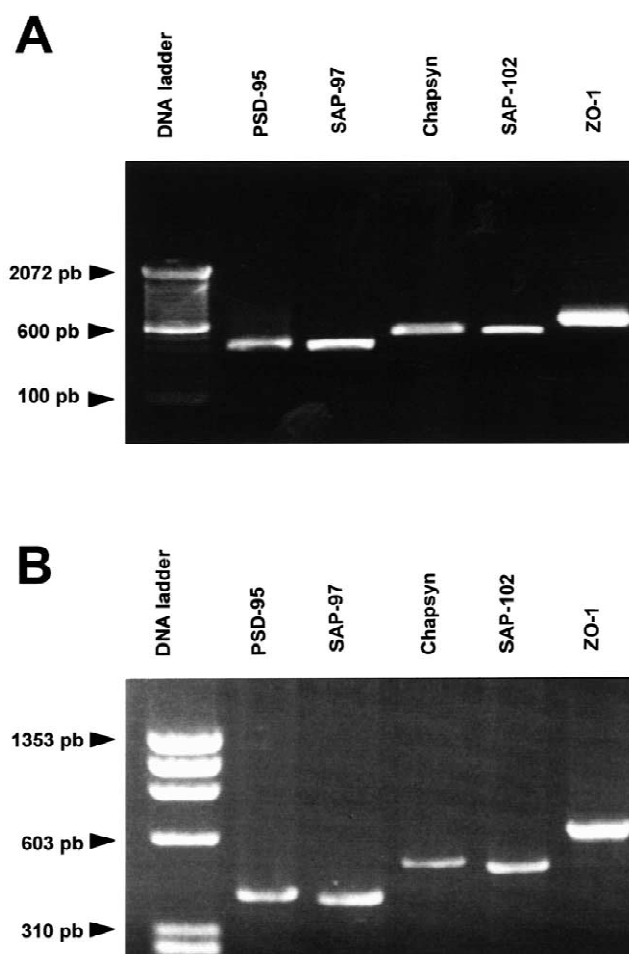


Fig. 1. Transcripts of several MAGUK proteins obtained by RT-PCR on mRNA extracted from human atrial myocardium (A) or from isolated myocytes (B) (annealing temperature: 55 °C; 30 cycles of amplification).

localized at the level of the intercalated disk (Fig. 3B, arrowhead).

### 3.2. Calpain-I cleaves MAGUK proteins

As shown in Fig. 4A, anti-PSD-95 family antibody probing of the cytosolic protein fraction identified a strong 80 kDa band but only a very weak doublet at 140 kDa (Fig. 4A). This 80 kDa protein was observed despite the use of phosphatase and protease inhibitors in the lysate buffer, and also after boiling the protein or using DTT as solvent. We then examined whether this 80-kDa band resulted from proteolytic cleavage of MAGUK protein by a calcium-dependent protease such as calpain-I that cleaves neuronal PSD-95 [25]. Indeed, calpain-I cleaved human atrial myocardium PSD-95 protein in a concentration- and  $\text{Ca}^{2+}$ -dependent manner (Fig. 4B). The 95 kDa doublet gradually vanished, while 80 kDa and then 46 and 36 kDa bands accumulated suggesting the unmasking of cleavage sites during proteolysis with increasing concentration of the protease. The same procedure led to a concentration-

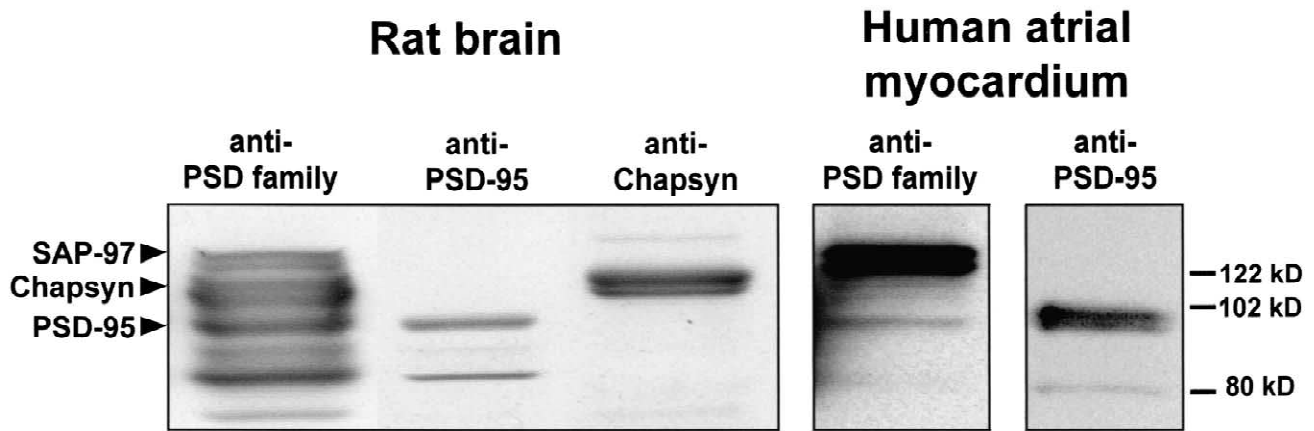


Fig. 2. Western blot analysis of MAGUK proteins expressed in human atrial myocardium. Rat brain protein, used as positive control, contained various MAGUK proteins that cross-reacted with anti-PSD-95 family, anti-PSD-95 and anti-Chapsyn antibodies. In protein from human atrial myocardium, the anti-PSD-95 family antibody detected an intense doublet at 140 kDa (identified as SAP-97 protein), together with a 95 kDa band (also detected with anti-PSD-95) and very weak bands at around 110 and 80 kDa.

dependent degradation of the 140-kDa protein, with accumulation of 95 and 80 kDa bands. At the highest concentrations tested, 65, 57, 46 and 36 kDa bands accumulated, the former two being detected only with the anti-PSD-95 family antibody (Fig. 4C and D). The cocktail of protease inhibitors suppressed this proteolytic process (not shown). These results indicated that SAP-97 was a substrate for calpain-I protease activity and that its cleavage may account for the cytosolic accumulation of a short 80 kDa form.

### 3.3. SAP-97 interacts with hKv1.5 shaker channels

To examine whether SAP-97 associated with hKv1.5 channels, we co-immunoprecipitated the two proteins. As

shown in Fig. 5A, the 140 kDa protein and, to a lesser extent, the 95 and 80 kDa proteins were detected in the protein fraction that was immunoprecipitated from MT by the anti-PSD-95 family antibody, while they were not detected in the supernatant (negative control consisted in incubating protein with beads that were not coated with antibody). Probing of the immunoprecipitate with anti-hKv1.5 channel antibody revealed the presence of a protein that migrated at around 70 kDa. Only a weak 70 kDa band was detected in the remaining supernatant protein, suggesting that a large proportion of the protein was co-precipitated with the MAGUKs (negative control consisted to probe the gel with only the secondary antibody). Further evidence for the interaction between SAP-97 and hKv1.5 channels was obtained by the detection, with anti-PSD-95

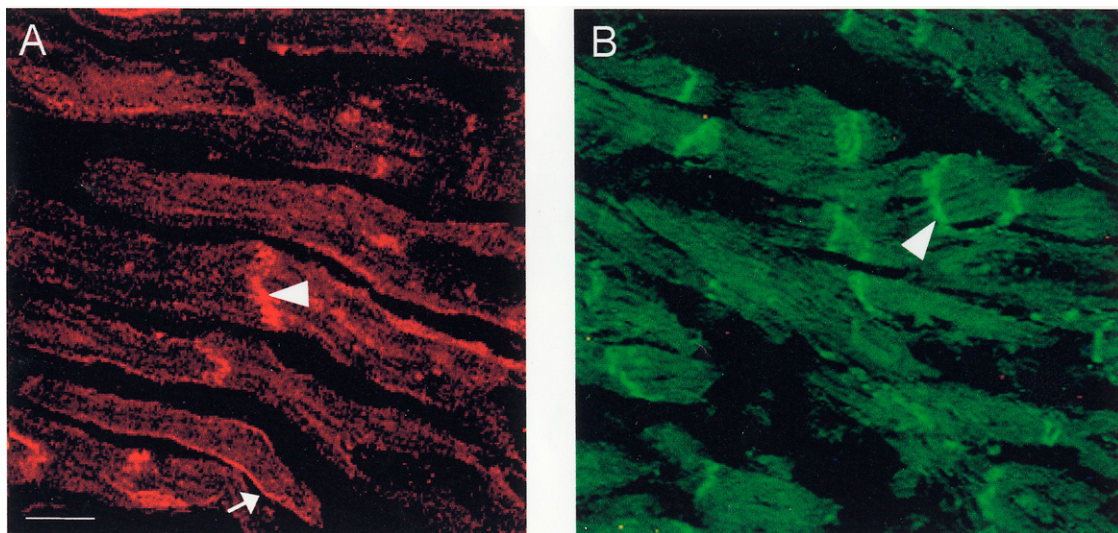


Fig. 3. Immunolocalization of PSD-95 family proteins (A) and hKv1.5 channels (B) in human atrial myocardium showing a staining that predominated at the level of intercalated disks (arrowheads). A staining at the periphery of myocytes (arrow) was also obtained with the anti-PSD-95 family proteins antibody. Bar = 10  $\mu$ M.



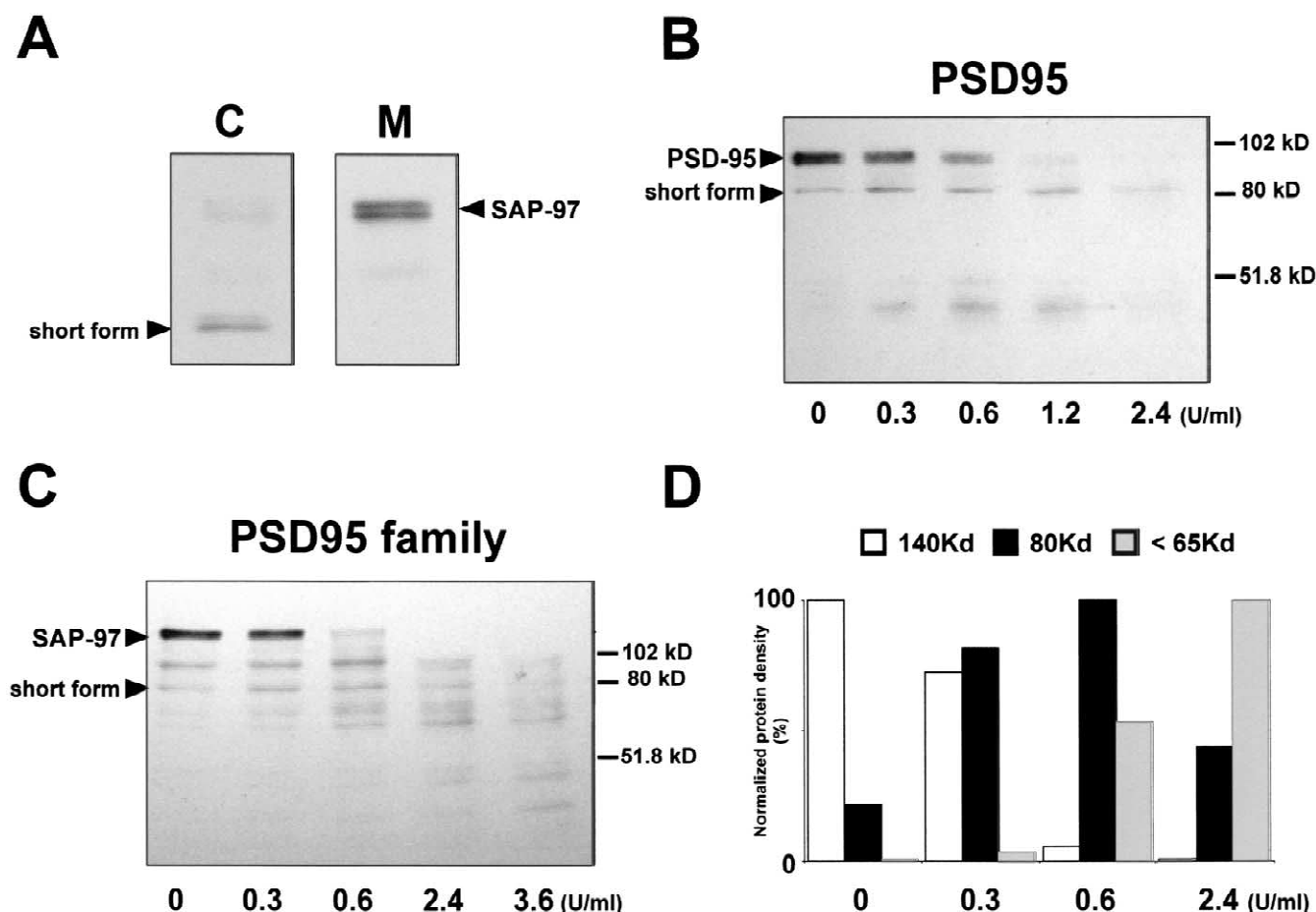


Fig. 4. Proteolytic cleavage of MAGUK proteins. (A) In the cytosolic protein fraction, C, anti-PSD-95 family antibody detected a strong band at 80 kDa and only a weak band at 140 kDa, contrasting with the pattern obtained with the membrane fraction, M. (B) Concentration-dependent effects of the calcium-dependent neutral protease calpain-I on proteins detected using the anti-PSD-95 (B) and anti-PSD-95 family (C). (D) Densitometric analysis of bands obtained upon SAP-97 cleavage by various concentration of calpain-I.

family antibody, of a strong 140 kDa band in the immunoprecipitate obtained with the two anti-hKv1.5 channel antibodies ( $n=3$ ) (Fig. 5B). Other bands, including one at 95 kDa were detected in the immunoprecipitate, suggesting that hKv1.5 channels interact with various MAGUKs.

### 3.4. SAP-97 increases the hKv1.5 current

To determine the functional consequences of the interaction between SAP-97 and hKv1.5 channels, the two proteins were co-expressed in CHO cells and currents were recorded by using the patch-clamp technique. An outward current with characteristics of the current carried by hKv1.5 shaker channels was detected in CHO cells expressing hKv1.5 either alone or together with SAP-97 protein, with a threshold activation potential at around  $-20$  mV, rapid and voltage-dependent activation, strong sensitivity to  $100 \mu\text{M}$  4-AP and insensitivity to  $30 \text{ mM}$  external TEA (not shown) (Fig. 6A). The reversal potential of the deactivation current was  $-82 \pm 2$  mV indicating a

high selectivity for  $\text{K}^+$  ( $E_k = -85$  mV). The density of the current recorded in CHO cells expressing both hKv1.5 ( $0.5 \mu\text{g}$ ) and SAP-97 ( $1.5 \mu\text{g}$ ) was higher than that recorded in cells expressing only hKv1.5; this was statistically significant at all potentials at which the current ( $157.00 \pm 19.45$  pA/pF vs.  $344.50 \pm 58.58$  pA/pF at  $+50$  mV;  $n=10$ ;  $P<0.05$ ; Fig. 6A, B and C). A same stimulatory effect of the SAP-97 on the current was observed with the following ratio MAGUK/Channel: 1:4, 1:15 and 1:150. When CHO cells were transfected with a very low concentration of hKv1.5-cDNA ( $0.01 \mu\text{g}$ ) the effect of the co expression of the SAP-97 ( $1.5 \mu\text{g}$ ) on the current was enhanced (at  $+50$  mV:  $99.07 \pm 17.70$  pA/pF,  $n=8$  vs.  $271.88 \pm 33.09$  pA/pF,  $n=8$ ,  $P<0.001$ ). In addition to increasing the current density, the co-expression of the hKv1.5 with the SAP-97 was associated with a shift of the activation-voltage relationship of the current towards positive potentials (Fig. 6D; Table 1). Taken together, these results indicated that SAP-97 protein modulated the functional expression of hKv1.5 channels.

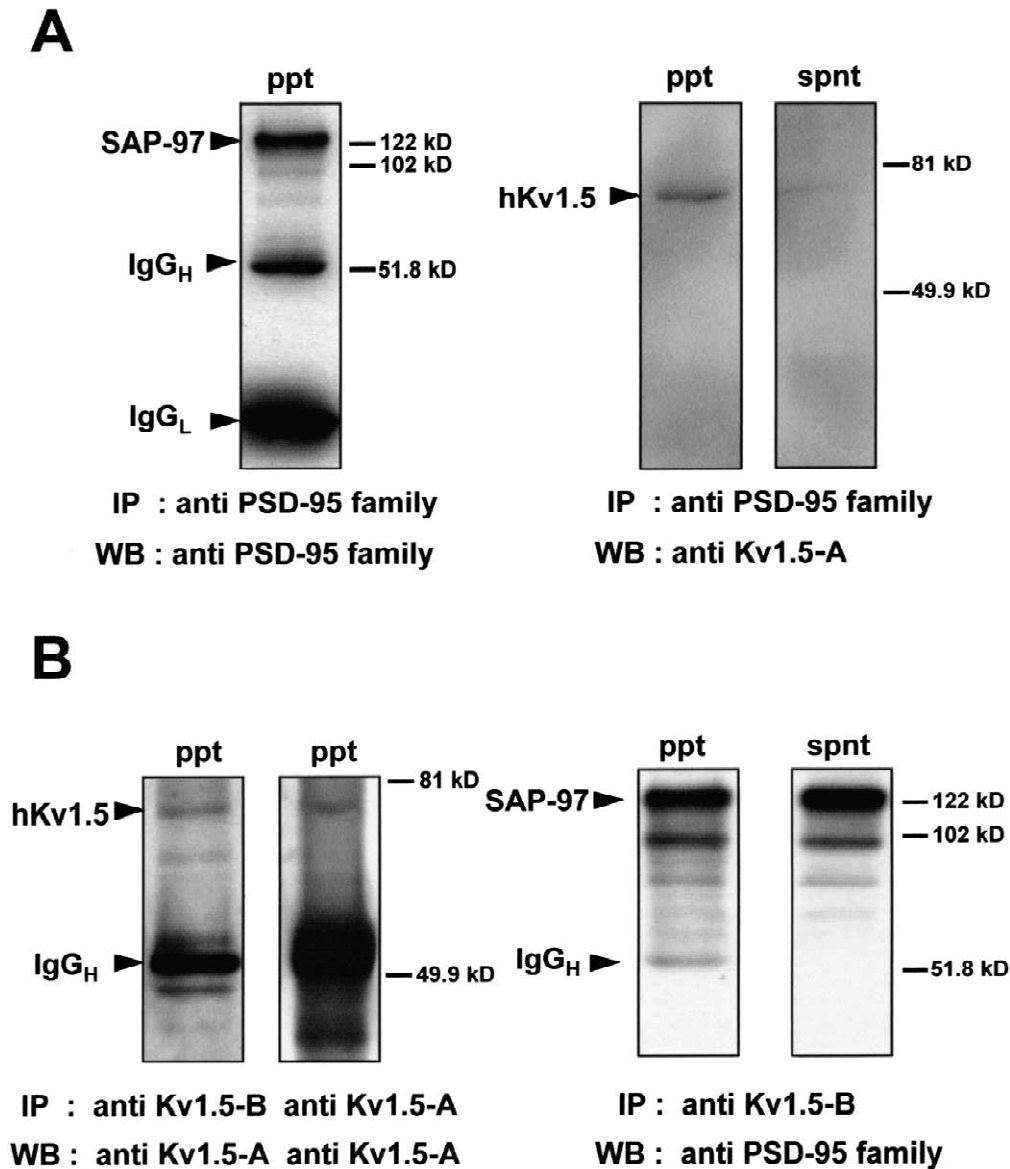


Fig. 5. Co-immunoprecipitation of SAP-97 and hKv1.5 shaker channels. (A) The atrial myocardial membrane protein fraction was immunoprecipitated with anti-PSD-95 family antibody, and Western blots were performed with the same MAGUK antibody and with the anti-Kv1.5-A channel antibody. (B) Proteins were immunoprecipitated with two distinct anti-Kv1.5 antibodies, and Western blot was performed with the anti-Kv1.5-A channel antibody (see Methods for more details) and the anti-PSD-95 family antibody. ppt, for precipitate; spnt, for supernatant.

#### 4. Discussion

To the best of our knowledge, this study is the first detailed characterization of the expression of the MAGUK proteins in human atrial myocardium. We found that SAP-97 and, to a lesser extent, PSD-95 are abundantly expressed in this tissue, whereas Chapsyn-110 and SAP-102 are barely detectable at the protein level, despite the presence of their messengers. This is consistent with previous studies indicating that SAP-97 is an ubiquitous protein but contrasts with reports that PSD-95 protein is restricted to the brain [2]. However, we cannot rule out contamination of our atrial myocardium preparations by

neuronal proteins. As in other tissues, SAP-97 migrated in SDS-gel as a doublet at around 140 kDa. This does not seem to be due to changes in phosphorylation status as phosphatase treatment had no effect (not shown). It may rather reflect the presence of different isoforms of SAP-97 as previously reported in other tissues [2,26].

Rat ventricular myocytes also contain SAP-97 protein, which predominates in the intercalated disk and T-tubule system, and co-localizes with Kir2.2 and Kv1.5 channels [12,16]. In human atrial myocytes too, MAGUK proteins are localized at the level of the plasma membrane predominantly at the intercalated disk. Moreover, we obtained direct biochemical evidence that SAP-97 forms complex

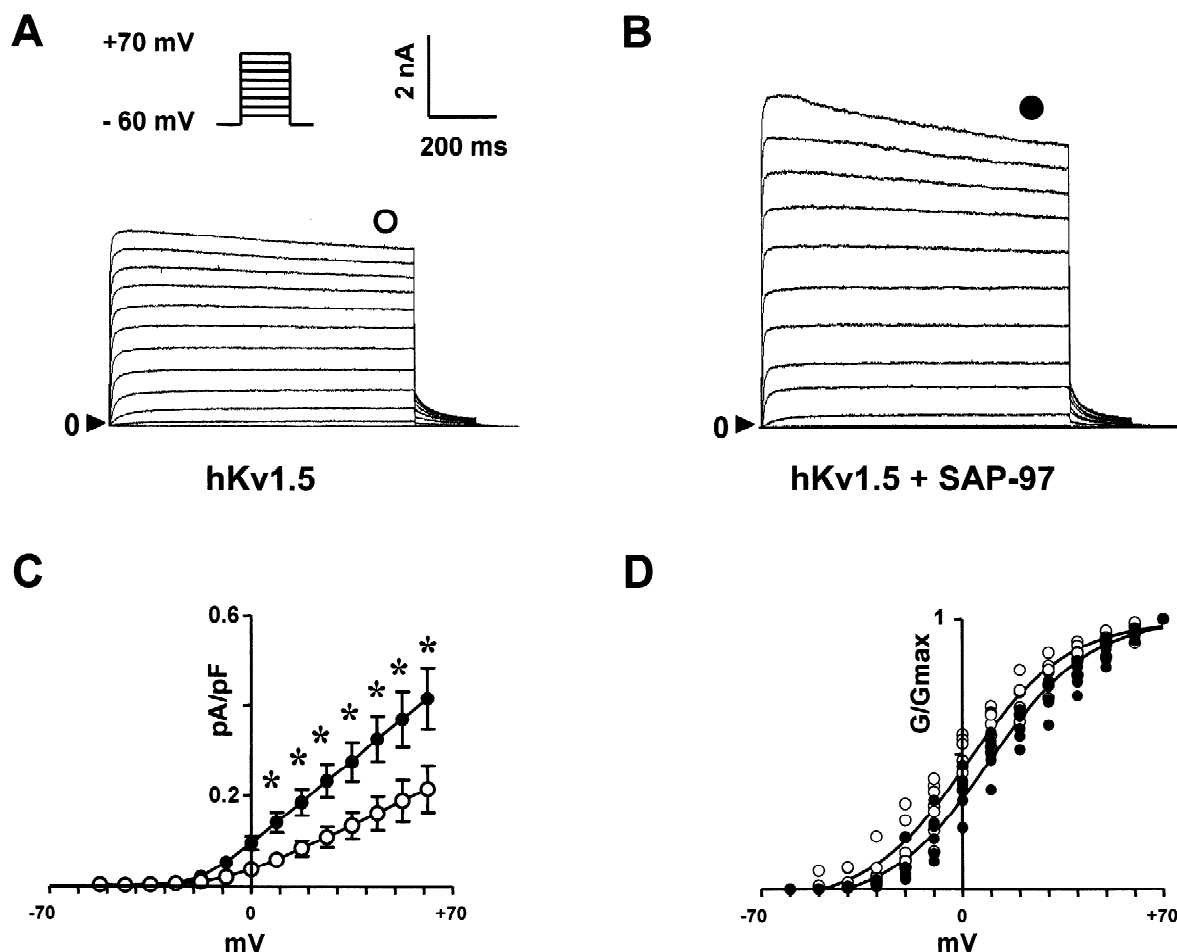


Fig. 6. Effect of SAP-97 on functional expression of hKv1.5 channels in CHO cells. (A) Traces of current elicited by 750-ms incremental test pulses starting at -60 mV in CHO cells expressing hKv1.5 channels alone or together with SAP-97 protein (B). Cell capacitance: 28 and 20 pF, respectively. C, current density–voltage relationship of the hKv1.5 current (○) and the hKv1.5/SAP-97 current (●); each point is the mean for eight to 10 cells. D, steady-state activation curve of CHO cells expressing hKv1.5 channels alone (○) or together with SAP-97 (●); normalized conductances are plotted as a function of the test potential ( $E_k = -85$  mV). \*  $P < 0.05$ .

with hKv1.5 *Shaker* potassium channel in human atrial myocardium. In their study, Murata et al. failed to coimmunoprecipitate the SAP-97 and Kv1.5 in rat ventricular myocardium presumably because of the low level of expression of Kv1.5 channel in this tissue. This is not the case of the human atrial myocardium where hKv1.5 channel is one of the main channels carrying the outward potassium current [17–19]. Interactions between MAGUK proteins and Kv channels have been described for *shaker* channels such as Kv1.1, Kv1.2 and Kv 1.3 and 1.4 [2]. These channels share, at their C-terminal end, a TDV motif

that is believed to mediate their interaction with the PDZ domains of MAGUK proteins. In hKv1.5 channels this amino acid sequence is replaced by TDL, which also permit binding to PDZ domains of MAGUK proteins [12]. Another evidence for the interaction between SAP-97 and the human Kv1.5 is provided by the observation that the functional expression of the hKv1.5 is enhanced by the MAGUK protein in CHO cell, as previously described for rat channels expressed in *Xenopus* oocytes [12].

Controversy exists in the literature concerning the capacity of SAP-97 to regulate  $K^+$  channel surface expression. It has been reported that, in Cos-1 cells, SAP-97-Kv1.1 *shaker* channel interaction leads to the formation of intracellular clusters whereas, in the same cells, PSD-95 enhances channel expression at the plasma membrane [14]. This contrasts with the promoting effect of SAP-97 on surface expression of Kir4.1 [10] and Kv channels in neurons [13]. Perhaps in some type of cells such as CHO cells, *Xenopus* oocytes and cardiac myocytes, SAP-97 is efficiently targeted to the plasma membrane compartment,

Table 1

Voltage-dependent activation of hKv1.5 channels expressed alone or with SAP-97

	$V_{1/2}$ (mV)	$k$	$G_{max}$ (nS)
hKv1.5	$1.8 \pm 1.4$	$19.1 \pm 1.7$	$25.6 \pm 5.0$
hKv1.5+SAP-97	$9.3 \pm 1.8^{**}$	$18.4 \pm 2.1$ NS	$46.5 \pm 4.9^*$

\*  $P < 0.05$ ; \*\*  $P < 0.01$ .



permitting its intracellular interaction with Kv channels, or a specific transport mechanism ensures the surface targeting of intracellularly formed SAP-97–hKv1.5 complexes. Indeed, an important feature of SAP-97 protein is its specific localization, at synapses in neurons and at cell–cell contacts in epithelial cells which has been attributed to the presence of an amino acid sequence at the N-terminal part of SAP-97, which is not found on other PSD-95 family proteins [8], and allowing its interaction with scaffolding proteins [27]. Immunohistochemical studies have shown that, in human atrial myocardium, hKv1.5 channels are concentrated at the level of the intercalated disc [20]. Although the hKv1.5 channel can interact directly with cytoskeleton proteins via its N-terminal part, as shown in the HEK cell line [28], it is conceivable that SAP-97 also contributes to the specific subcellular localization of hKv1.5 channels in human myocardium. The shift in the voltage-dependent activation of hKv1.5 current that we observed when channels were co-expressed with SAP-97 may be another indication that this anchoring protein clusters  $K^+$  channels in membrane regions with distinct protein and lipid compositions that could modulate their gating properties [29,30].

In the cytosol of human atrial cells, we consistently detected an 80 kDa protein that cross-reacted with the anti-PSD-95 family antibody, which is directed against the PDZ-1 and -2 domains, suggesting that the 80 kDa band may correspond to a truncated N-terminal form of SAP-97. The truncation could result either from proteolysis by a protease whose activity is not inhibited by the protease inhibitors we used during the extraction procedure, or proteolysis occurring *in vivo* after activation of an endogenous protease. In neurons, proteolytic cleavage of PSD-95 by calpain-I is associated with synaptic plasticity during ontogenic development [26]. In atrial myocardium too, calpain-I cleaves SAP-97 and PSD-95 proteins, resulting in accumulation of short forms, including an 80 kDa band that might correspond to the ‘short’ MAGUK protein found in the cytosol. The significance of this proteolytic truncation of MAGUK proteins remains to be determined, together with its consequences for cardiac electrical activity with enhanced calpain activity.

In summary, this study identifies novel partners for ionic channels in human atrial myocytes, that could play an important role in the cell–surface expression and clustering of these channels. In other cell types, these anchoring proteins also contribute to channel regulation by second messengers. For instance, regulation of the NMDA receptor by nNOS [31] and  $\alpha$ CAMK-II [32] is facilitated by the assembly of protein networks by MAGUK proteins. Likewise, SAP-97 sensitizes Kir3.2c potassium channels to G protein stimulation [15]. In human atrial myocardium, the activity of the channels carrying the outward  $K^+$  current is regulated by various second messengers [33] and this regulatory process may also be facilitated by MAGUK proteins and by the constitution of an appropriate channel

environment. This system could be an important new element in the regulation and adaptation of myocardial electrical properties.

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