Sap97 Expression and Autonomic Regulation of Heart Rate

Aoun K*, Ulbrick M*, Ramos Mondragon R, Rosinski B, Musa H, Guerrero-Serna G, Valdivia H and Anumonwo J

Departments of Internal Medicine (Cardiovascular) and of Molecular and Integrative Physiology
University of Michigan, Ann Arbor, MI

(* equal contributions)

ABSTRACT

Background
Synapse-associated protein 97 (Sap97) is a membrane associated guanylate kinase like protein that clusters ion channels, beta-1 adrenergic receptor (β1-AR) and enzymes. In the heart, such macromolecular complexes of proteins are important for regulating electrical impulses. We have examined the role of *Sap97* deletion in beta-adrenergic regulation of the murine heart rate (HR).

Methods and Results
A murine model of cardiac targeted *Sap97* gene deletion was generated using the Cre-Lox system. Standard electrophysiological and biochemical techniques were used for investigations in wild type (WT) and Sap97 knockout (*Sap97* KO) animals. ECGs were carried out on anesthetized WT and *Sap97* KO animals under control conditions and following isoproterenol (ISO) challenge (1.5 mg/kg; SC). In WT mice, with ISO challenge, there was an increase in HR that returned to baseline with a biphasic profile; an initial rapid decline (<5 mins) and a longer lasting decline to baseline (~20 mins). *Sap97* KO animals also showed an initial rapid decline in HR but a more prolonged decline, which remained at 25% above baseline (N=3,3). In a different set of mice, telemetry was performed on conscious animals, which were challenged with ISO after *Sap97* ablation (N=4). HR in the mice showed a similar biphasic response, remaining 35% above baseline after *Sap97* KO (p = 0.047). Tissue samples were isolated from the sinus node region in WT and *Sap97*KO mice to study molecular mechanisms that may underlie the changes in HR. In *Sap97* KO animals, compared to WT, protein levels (relative to GAPDH) showed ~86% reduction in Sap97 protein from 0.7+/- 0.1 to 0.1+/- 0.05 (p=0.008). In contrast, there was 133% increase in β1-AR protein, from 0.3+/- 0.1 to 0.7+/- 0.1 (p=0.003). Whereas HCN4, a molecular correlate of the pacemaker channel was decreased in *Sap97* KO cells, protein kinase A (PKA) and Cav1.2 protein (L-type calcium channel protein) levels were relatively unchanged (N = 3-6). Single-cell experiments showed no differences in L-type calcium currents in WT and *Sap97* KO animals.

Conclusion
Results of our experiments in the model of targeted *Sap97* ablation suggest that *Sap97* expression is important for autonomic regulation of HR. Our results also suggest a regulatory mechanism that is dependent on β1-AR, but independent of the HCN4 and Cav1.2 protein levels.

BACKGROUND

Sap97

- **Membrane Associated Guanylate Kinase**
- **Multi-domain protein**
- **Defined as a MAGUK because of the GUK domain**
- **PDZ and SH3 domains**
  - **Protein-protein interactions**
- **L27 domain**
  - **β-SAP97**
  - **dimerization**
- **Archetypal scaffolding protein**

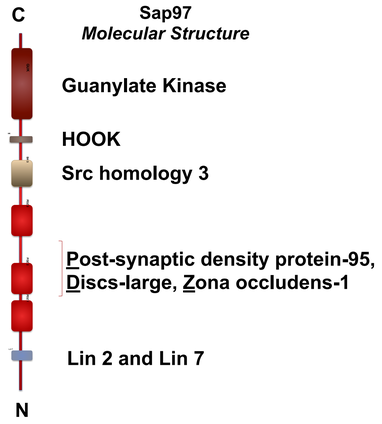

Figure 1. Functional domains of Sap97

Current	Protein	C-term
I_{K1}	Kir2.1 Kir2.2 Kir2.3	PRPLRRESEI ERPYRRESEI NISYRRESAI
I_f	HCN2 HCN4	SARSRLSSNL PVRSKLPSNL
I_{to}	Kv 4.2 Kv 4.3	GGNIVRVSAL TSNVVKVSAL
$I_{Ca,L}$	Cav1.2	ADSRSYVSNL
I_{Na}	Nav1.5	SPDRDRESIV
$I_{K\ slow}$	Kv1.5 Kv2.1	CLDTSRETDL DLLAILPYYV
---	β_1-AR	RQGFSSESKV

Table 1. Major cardiac ion channels/receptors have class 1 PDZ binding motif: S/T-X- Φ (S=Serine, T=Threonine, X = any amino acid;Φ = hydrophobic amino acid).

METHODS

- CRE-LOX Recombination for Model Generation

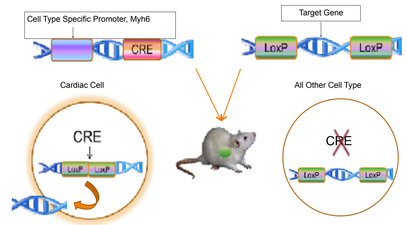

Figure 2.
Tamoxifen-inducible Cre-mediated recombination is expected to result in deletion of the floxed sequences [Exon encoding part of PDZ1 and PDZ2 domain of SAP97] in heart cells of the offspring.

- Echocardiography and Electrocardiography

  Whitesall et al, 2004.

- Cell Electrophysiology and Biochemistry

  Vaidyanathan et al, 2010.

  Milstein et al, 2012.

  Musa et al, 2013.

Generating Sap97 KO Model

- Mice: Two stains used for **inducible**, **cardiac-specific**, KO model
- Strain #1: alpha-MHC MerCreMer transgene (*Myh6* promoter directs the expression of tamoxifen-inducible Cre recombinase)
- Strain #2. *loxP* sites on either side of exon encoding PDZ1,2
- Mating and offspring genotyping
- **Controls (4): *Sap97$^{wt/wt}$*, *Sap97$^{wt/cre}$*, *Sap97$^{wt/fl}$* and *Sap97$^{fl/fl}$***
- **Test: *Sap97$^{cre;\ wt/fl}$* (heterozygote)**
  Sap97$^{cre;\ fl/fl}$ (homozygote)
- Tamoxifen injection (5-days)
- Experiments >2 weeks

RESULTS

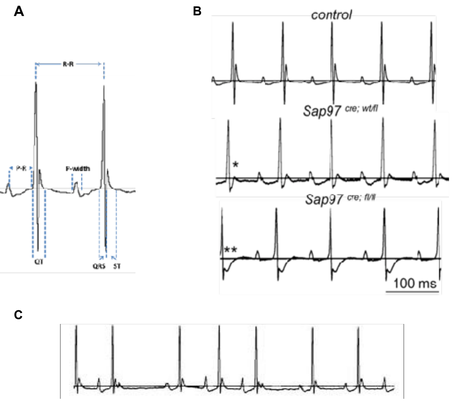

Figure 3. ECG properties in wild type and in *Sap97* KO mice
A. Schematic representing details for analysis of EKG waveforms. B, C. ECGs in wild type and KO animals. Note typical changes in ST of hetero- and homo-zygote (respectively, one and two asterisks) Sap97 KO animals. Note the profound delayed repolarization effects in the ECG in homozygote KO. Abnormal atrial electrical activity is shown in C.

TABLE 2

HET	RR (ms)	ST (ms)	QRS (ms)	PR (ms)	QT (ms)	P-width (ms)
Control (N=7)	113.37	15.9	8.4	40.0	23.7	10.8
S.E.	3.98	0.98	0.30	0.96	0.99	0.99
SAP97$^{cre;wt/fl}$ (N=6)	115.19	22.3	8.7	41.9	30.6	14.1
S.E.	6.85	2.53	0.21	1.04	2.48	1.51
P-values	0.81	0.03*	0.42	0.18	0.02*	0.08

TABLE 3

HMZ	RR (ms)	ST (ms)	QRS (ms)	PR (ms)	QT (ms)	P-width (ms)
Control (N=13)	108.7	15.5	8.7	41.7	23.9	11.4
S.E.	2.80	2.22	0.26	1.13	2.19	0.55
SAP97$^{cre;fl/fl}$ (N=13)	112.7	34.8	9.4	41.3	43.7	13.0
S.E.	4.19	3.61	0.35	1.21	3.46	0.72
P-values	0.5	.0001*	0.2	0.8	.00001*	0.14

Tables 2&3. ECG measurements in WT and *Sap97 KO* animals ECG (lead II; anesthetized) analysis in wild type and heterozygote (Table 2) and homozygote (Table 3) mice.

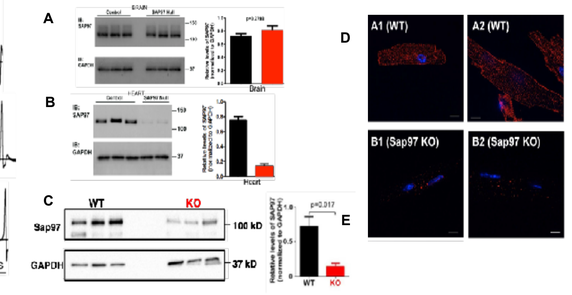

Figure 4. Targeted deletion of *Sap97* in a murine model A. Sap97 expression in the brain. B. Sap97 expression in the ventricular tissue. C. Sap97 expression in the atrial tissue. Protein quantification data are shown as histograms. Immunofluorescence of Sap97 in atrial (A1) and ventricular (A2) cells in control (D) and in KO (E).

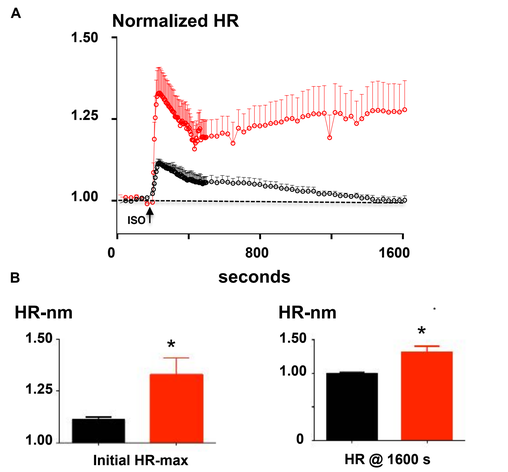

Figure 5. Isoproterenol effects on heart rate in WT and *Sap97* KO mice A. Heart rate measurements (Lead II ECG) in anesthetized (1.5% isoflurane) mice. B. Heart rate measurements in conscious mice before (black) and after (red), tamoxifen injection for cardiac specific ablation of *Sap97*. Normalized HR as shown in the graphs; baseline absolute values and normalized HR are shown in the bar graphs.

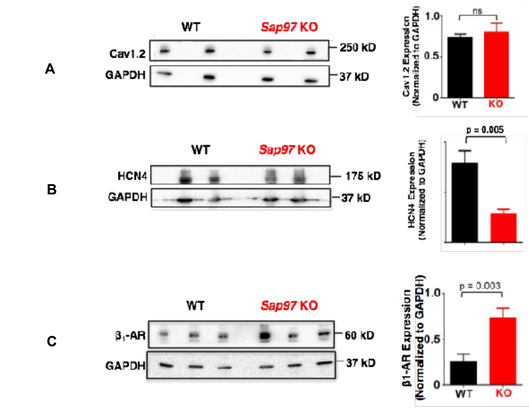

Figure 6. Western Blot analyses of ion channels and beta1-adrenergic receptor in mouse atria A. WB conducted on atrial tissue lysates from wild type and *Sap97* KO animals, for Cav1.2 (A), HCN4 (B) and beta1-adrenergic receptor proteins (C). Protein expression normalized to GAPDH show differential effects on ion channels and receptors following *Sap97* ablation.

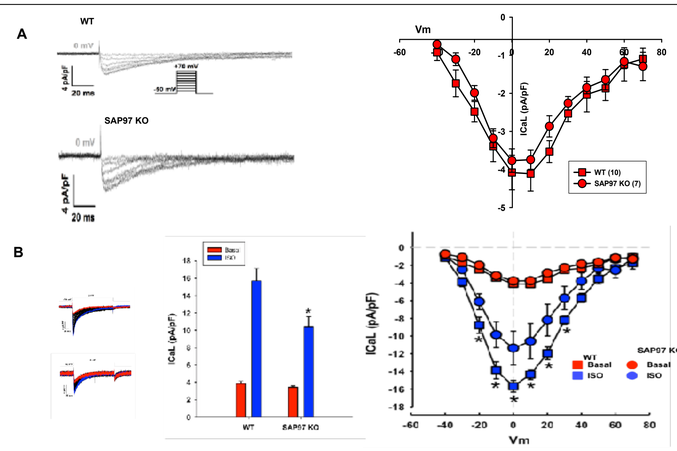

Figure 7. Adrenergic regulation of L-type calcium current in WT and in Sap97 KO cardiac myocytes. A. Cav1.2 expression is unaltered by Sap97 KO. B. Differential sensitivity to isoproterenol (ISO) of WT and Sap97 KO cardiac myocytes.

SUMMARY AND CONCLUSIONS

- Generated a murine, inducible cardiac specific *Sap97* knockout (KO) model
- ECGs in *Sap97* KO mice have abnormalities in atrial and ventricular electrical excitation
- Heart rate in wild type and *Sap97* KO animals differentially responded to isoproterenol challenge
- In atrial tissue of *Sap97* KO mice compared to wild type:
  - HCN4 expression was down regulated
  - Cav1.2 expression was unchanged
  - β_1-AR expression was upregulated

Sap97 expression plays a role in the normal assembly of a macromolecular signaling complex involving of β_1-AR and cardiac ion channels

Sap97 expression is important for β_1-AR regulation of the electrical impulse in the murine myocardium

ACKNOWLEDGEMENTS

Supported by:

1R01HL124319 (National Institutes of Health)

14GRNT19710006 (American Heart Association)

REFERENCES

- Milstein ML, Musa H, Balbuena DP, Anumonwo JM et al. Proc. Natl. Acad. Sci U S A. 2012.
- Musa H, Carleton L, O'Connell R , Gomez et al. Circulation. 2013.
- Vaidyanathan R, Taffet SM, Vikstrom KL, Anumonwo JM. J Biol. Chem. 2010.
- Whitesall S, Hoff JB, Vollmer AP and D'Alecy L. Am J Physiol Heart Circ Physiol. 2004.