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Abstract 7611

G_q Signaling-Induced Ca^{2+} Waves Abolished by TRPC Inhibition

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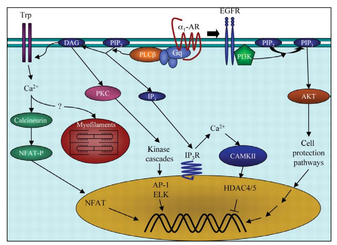
INTRODUCTION

Atrial fibrillation (AF) and congestive heart failure (HF) frequently coexist, preempting optimal treatment of either. While the causes of AF and HF are multi-factorial, a common molecular level occurrence in HF is the upregulation of G_q signaling consequent to hypertension, which if chronic leads to hypertrophy and eventually HF. How G_q signaling relates to the development of AF is unknown. Increased intracellular calcium (Ca^{2+}) is key in both G_q-mediated hypertrophy, and in proarrhythmic activity such as Ca^{2+} waves. Canonical transient receptor potential channels (TRPCs) are Ca^{2+}-permeable nonselective cation channels activated by G_q-signaling, and thus provide a means by which G_q signaling can bring about both hypertrophy and proarrhythmic activity. We have begun studies to determine which TRPCs are expressed in atria, and if those identified play a role in Ca^{2+}-mediated proarrhythmic activity. If so, TRPC channels may represent novel antiarrhythmic targets, particularly in the cases of AF coexistent with CHF.



Phenylephrine Utilizes the α1-Adrenergic Receptor to Activate the G_q Pathway[1]

MATERIALS AND METHODS

Isolated Myocytes: Canine atrial myocytes were isolated as previously described.[2]

RT-PCR: Reverse Transcription - Polymerase Chain Reaction was performed with RNA isolated from Canine and Human posterior left atrium tissue using the Qiagen RNeasy® Fibrous Tissue Mini Kit (Cat. No. 74704). TRPC Canine primer s were designed using Primer Quest℠ on www.idtdna.com. TRPC Human primer sequences were obtained from Paria (2004).[3] RT product was amplified for 35 cycles using *Taq* polymerase from Promega (Cat. No. M7502). The amplified DNA was visualized using Lonza's Flash Gel System (Cat. No. 57026).

Western Blot: Membrane proteins were isolated from Canine atrial tissue using ProteoJET™ Membrane Protein Extraction Kit from Fermentas (Cat. No. K0321), separated on a 10% SDS-Page gel, and transferred to a PVDF membrane. G_q rabbit polyclonal antibody was obtained from Upstate (Cat. No. 06-709); and the TRPC3 rabbit polyclonal antibody was obtained from AbCam (Cat. No. ab51560). Equal loading was assessed and bands normalized using Pierce Reversible Protein Stain Kit for PVDF Membranes (MemCode) (Cat. No. 24585).

Acquisition of Ca transients: Real-time confocal fluorescence measurements of Ca^{2+} transients in isolated atrial myocytes were as modified from previous studies[2]. Briefly, aliquots of cells were loaded with 15-20 μM of the non-ratiometric Ca^{2+} fluorescence dye, fluo-4AM (Molecular Probes, Invitrogen) from stocks of 1mM fluo-4-AM dissolved in DMSO containing 20% pluronic acid, for 20-40 min. The cells were then placed in a field stimulation cell chamber mounted on the stage of an inverted laser scanning confocal microscope (Axiovert 100-LSM 510 system: Carl Zeiss) and washed via bath superfusion with normal Tyrode's solution for 10-15 min. Normal Tyrode's bath superfusion was continued for the duration of the experiment while the cells were electric field stimulated at 0.5 Hz. Only cells that had smooth membranes, clear striations and responded to field stimulation with detectable (by eye) contractions were deemed competent myocytes. All isolated myocyte experiments were conducted at room temperature (20-25 C).

Confocal X-t line-scan recordings of uncalibrated fluo-4 fluorescence (excitation: 488 nm Argon laser line; collected emission: >505 nm longpass filtered) of the evoked cellular Ca^{2+} transients were acquired using the integrated Zeiss LSM Confocal Microscopy Software (V2.5) and either a 25x (NA 0.82, max. spatial resolution is ~0.3 μm at the 517 nm emission maxima for fluo-4) or 40x (NA 1.24, max. spatial resolution is ~0.2 μm at the 517 nm emission maxima for fluo-4) water objective at a typical pixel density of 512 pixels/line and a scan rate of 1.92 msec/line-scan. Photobleaching and phototoxicity by laser light was minimized by scanning at < 10% output transmission for ≤ 30 s.

Control (normal Tyrode's), PE-containing, BTP2 –containing and PYR3-containing solutions were superfused focally to individual cells for stated durations via a micromanipulator positioned multi-line micromanifold (8 into 1) superfusion apparatus (MPR8, Cell Micro).

Drugs:

Drug	Full Name	Target
PE	Phenylephrine	Activation of a1-adrenergic receptors coupled to G_q
BTP2	4-methyl-4′-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide	Non-selective TRPC channel blocker
PYR3	ethyl-1-(4-(2,3,3-trichloroacrylamide)phenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate	Selective TRPC3 channel blocker

RESULTS

Figure 1. TRPC Channel Gene Expression

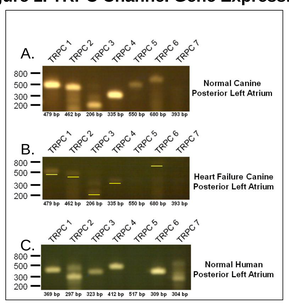


Figure 2. TRPC3 and G_q Protein Expression

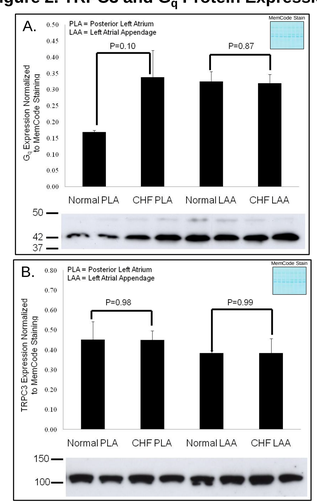


Figure 3. Phenylephrine Evoked Ca^{2+} Waves in Atrial Myocytes Are Inhibited by BTP2 - a Non-Selective TRPC Channel Blocker.

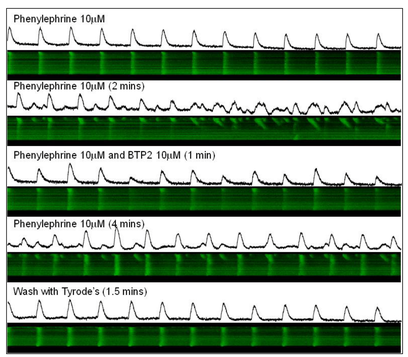


Figure 4. Phenylephrine Evoked Ca^{2+} Waves in Atrial Myocytes Are Also Inhibited by PYR3 - a Selective TRPC3 Channel Blocker.

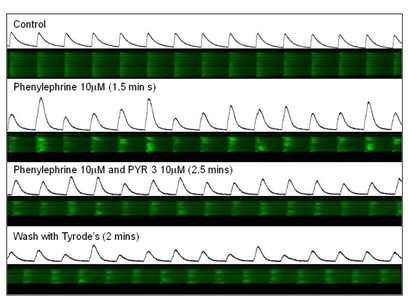


Figure 5. PYR3 Reduces the Ca^{2+} Transient As Well As Inhibited Phenylephrine Evoked Ca^{2+} Waves in Atrial Myocytes.

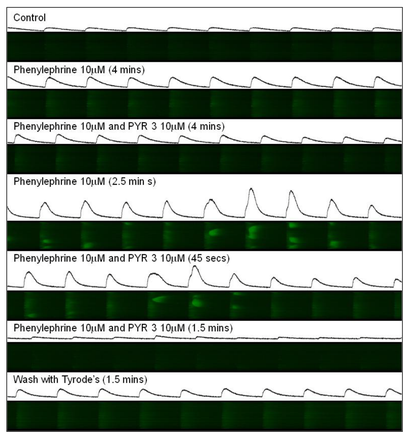
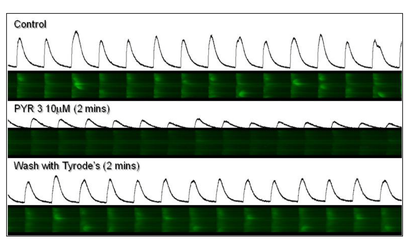


Figure 6. PYR3 Alone Inhibits Spontaneous Ca^{2+} Waves in Untreated Atrial Myocytes



DISCUSSION

- Message for TRPC isoforms 1, 3, 4, and 6 are present in both Canine and Human Atrial Tissue (Figure 1). TRPC2 is a pseudogene in humans and thus does not express protein.
- G_q protein increases approximately two fold in Canine PLA compared in heart failure compared to normal (Figure 2A).
- TRPC3 protein is present in Canine PLA, but does not increase in heart failure (Figure 2B). Increased TRPC3 channel activity could be explained by the increase in G_q protein (Figure 2A).
- BTP2 reversibly inhibits Ca^{2+} wave production induced by PE (Figure 3) indicating the family of TRPC channels are important in Ca^{2+} wave generation.
- The inhibition of PE induced Ca^{2+} waves by PYR3 (Figures 4 & 5) suggest that TRPC3 is a specific isoform of the TRPC family that is important in Ca^{2+} wave generation. However, TRPC3 also caused a decrease in the Ca^{2+} transient when compared to transients generated in control Tyrode's solution (Figure 5), which may also suggest that TRPC3 is involved in generating the normal Ca^{2+} transient.
- In normal myocytes that spontaneously generate Ca^{2+} waves without drug activation, PYR3 reversibly inhibits those Ca^{2+} waves (Figure 6) signifying that TRPC3 may be involved in spontaneous Ca^{2+} wave generation.

CONCLUSIONS

Gq-signaling in atrial myocytes can induce Ca^{2+}-mediated arrhythmogenic activity, which can be abolished by inhibition of TRPC channels, perhaps largely via TrpC3.

However, the attenuation of Ca^{2+} transients by TRPC antagonist alone indicates either or all of the following possibilities:

1. TRPC channels themselves significantly contribute to the maintenance of Ca^{2+} transients in atrial myoyctes.
2. TRPC channels functionally couple to other channels or Ca^{2+}-handling proteins in myocytes that contribute to the maintenance of atrial Ca^{2+} transients/E-C coupling.

REFERENCES

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[3]Paria BC. et al. *Am J Physiol Lung Cell Mol Physiol* (2004); 287(6):L1303-13.

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