The Role of Store Operated Calcium Entry and Transient Receptor Potential Channels in Atrial Fibrillation

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ABSTRACT

As the population ages, the incidence of atrial fibrillation (AF) will increase, representing a major health care burden. Myocytes from patients with AF have abnormal calcium signaling that may result from inositol-1,4,5-triphosphate (IP_3) mediated store operated calcium entry. Transient receptor potential (TRP) channels are cation channels known to be activated by G_q/IP_3 signaling. Studies show 2 families of TRP channels, TRP canonical (TRPC) and TRP Melastatin (TRPM), are implicated in the development of arrhythmogenic calcium activity. In addition, members of the TRPC family are associated with stretch activated channels and hypertrophic gene expression during congestive heart failure, one of AF's most serious complications. Store operated calcium entry and TRP channels provide new targets for therapeutic intervention of abnormal calcium signaling that occurs during AF.

INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia and is characterized by abnormal electrical pathways that cause the atria to contract in an unorganized fashion, or fibrillate.[1] Close to 2.5 million adults are projected to have AF in 2010. The mortality rate for AF, as either the primary or underlying cause, has been increasing for the past 2 decades. The American Heart Association reports that the number of patients diagnosed with AF in the United States is projected to increase almost 5 fold in the next 40 years.[2] This will represent a significant health care burden. In 2005 alone, the estimated cost of treatment including hospitalization, in- and out- physician care, and medications was $6.65 billion.[1]

CALCIUM AND ATRIAL FIBRILLATION

Calcium has been theorized to have an important role in the development and perpetuation of AF. Calcium is already known to be involved in normal excitation-contraction coupling of atrial myocytes. The increased heart rate produced by AF causes electrophysiological remodeling in atrial cells that initiates a positive feedback loop in which AF begets more AF. Some of the electrophysiological changes are thought to result in abnormal calcium signaling. To determine if calcium abnormalities were present in patients with chronic AF, Van Wagoner et al isolated atrial myocytes from the right atrial appendage from patients with a normal sinus rhythm prior to cardiovascular surgery, non-failing organ donor hearts with normal sinus rhythm, and patients with chronic AF. Patch clamping techniques were used to analyze the calcium current through L-type calcium channels. They found almost one-third the calcium current through L-type calcium channels in patients with chronic AF compared to those with a normal sinus rhythm. However, in response to β-adrenergic stimulation with isoproterenol, the calcium current in the atrial myocytes from the chronic AF patients went up 4.3 fold, which is significantly higher than the 2.8 fold increase seen in atrial myocytes from normal sinus rhythm patients. In addition, they found that normal sinus rhythm patients about to undergo valve repair whose myocytes had higher L-type calcium current were also more likely to experience postoperative AF.[3] Therefore, when atrial myocytes are overloaded with calcium, they may contribute to arrythmogenesis. On the other hand, after AF has been established, there may be an adaptive response that reduces the number of basally active channels, which can be activated with β-adrenergic stimulation.

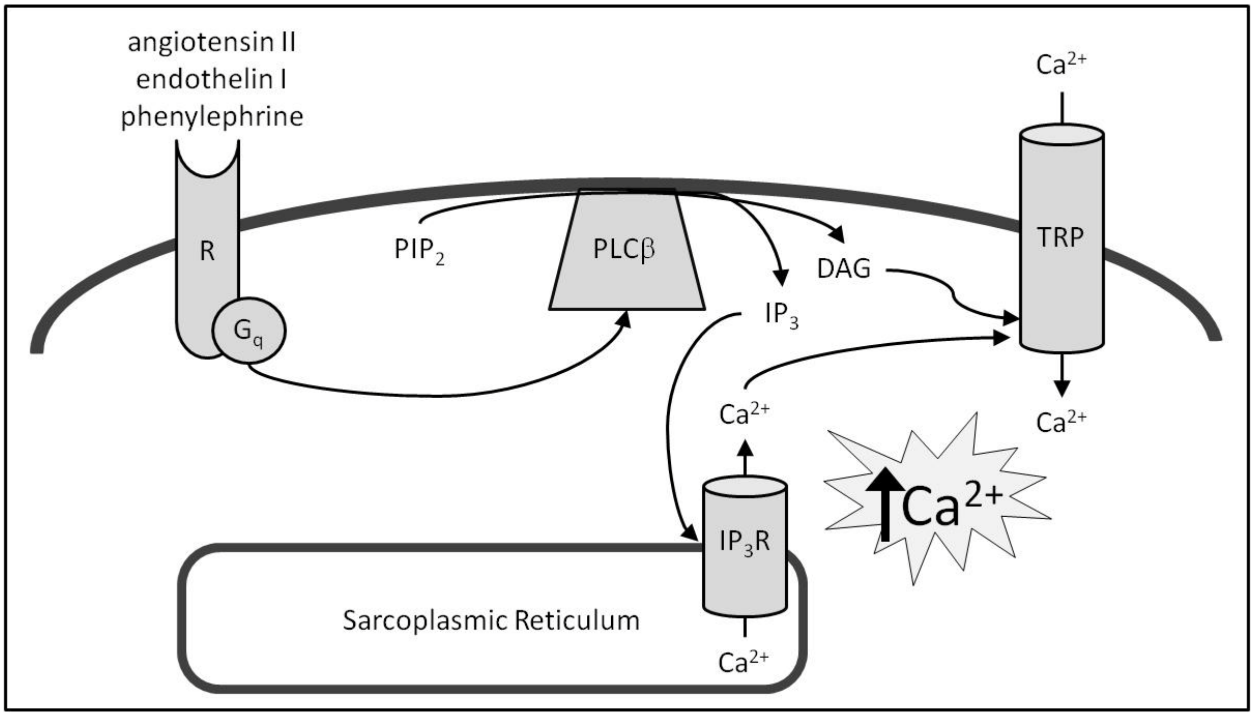
Not only does intracellular calcium concentration appear to be important in the development of AF, but the location from where that calcium comes may be crucial as well. Another group studied atrial myocytes from the right atrial appendage of patients with a history of AF as well as those who did not. They found that atrial myocytes from patients with a history of AF had more than twice the amount of calcium sparks and waves. Calcium sparks and waves are thought to be arrhythmogenic. They attributed these phenomena to sarcoplasmic calcium release since the calcium sparks and waves were inhibited when they preincubated the myocytes with the sarcoplasmic calcium pump inhibitor cyclopiazonic acid.[4] Therefore, calcium from the sarcoplasmic reticulum, the calcium store of the myocytes, may play a role in the development and maintenance of AF.

Pharmaceutical interventions are already being used to control calcium signaling during AF. In 1996, Goette and colleagues used the canine model to show that use of verapamil, an L-type calcium channel blocker, prevented the electrical remodeling and 12% shortening of the effective and absolute refractory periods seen in the rapidly paced control animal. When they added back calcium to verapamil infused animals they found the shortening similar to that seen in control dogs.[5] As indicated, verapamil is utilized as a treatment for abnormal heart rhythms in patients. However, the L-type calcium channel is an important component in normal excitation-contraction coupling. A better target for pharmaceutical intervention would be the abnormal calcium signaling present in AF.

STORE OPERATED CALCIUM ENTRY

One method for raising intracellular calcium concentration that does not involve normal excitation-contraction coupling is via store operated calcium entry. The figure shows the store operated calcium entry pathway. Agonists such as angiotensin II, endothelin I, and phenylephrine activate G_q coupled receptors.[6-8] Utilizing freshly isolated human atrial myocytes, 1 lab found that angiotensin II was responsible for a

Figure. Store Operated Calcium Entry Pathway



During store operated calcium entry, agonists such as angiotensin II, endothelin I, and phenylephrine stimulate their respective receptors (R) to release the G protein G_q to stimulate phospholipase C β (PLCβ) to convert phosphotidylinositol-4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3). IP_3 stimulates its own receptor (IP_3R) to release calcium (Ca^{2+}) from the sarcoplasmic reticulum. The cytosolic Ca^{2+} and DAG then stimulates transient receptor potential (TRP) channels to allow even more Ca^{2+} into the cytosol.

3-fold increase in calcium sparks after treatment when compared to control cells.[9] In the myocardium of the human heart, G_q activates the membrane-bound enzyme phospholipase C β to hydrolyze phosphotidylinositol-4,5-bisphosphate (PIP_2) to produce diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3).[10] IP_3 then diffuses through the cytosol and binds the IP_3 receptor on the sarcoplasmic reticulum allowing calcium to escape.[11] The escaped calcium in the cytosol, as well as DAG, can then stimulate calcium entry through transient receptor potential (TRP) channels on the plasma membrane.[12]

Previous studies have already shown that components of the store operated calcium entry pathway are involved in abnormal calcium signaling and AF. In rat atrial myocytes, diastolic, premature extra calcium transients were induced using a membrane permeable IP_3 ester that directly activates the IP_3 receptor, which was similar in the number of these transients induced by endothelin I in the same experiment.[13] Thus, they showed that endothelin I may utilize the store operated calcium entry pathway to induce arrhythmogenic calcium activity. Further, In IP_3 receptor deficient mice, endothelin I failed to cause an increase in the amplitude of the calcium transient and spontaneous calcium release as compared to wild type atrial myocytes.[14] Also, protein expression of IP_3 receptor in the atria increases with age in the rat.[15] Incidence of AF is known to increase with age in human patients. Finally, in the presence of tetracaine, a ryanodine receptor inhibitor, isolated atrial myocytes from the right atrial appendage of patients with AF had IP_3 induced calcium events with a 50% greater frequency and an approximately 50% longer time to half relaxation than in myocytes from patients with a normal sinus rhythm.[16] Store operated calcium entry is known to produce a sustained increase in intracellular calcium concentration as opposed to the transient increase produced through normal excitation-contraction coupling.

These studies provide evidence that calcium is an important player in the development and continuation of AF. Calcium overload of atrial myocytes contribute to arrhythmogenic activity such as calcium waves and sparks. Agonists that stimulate receptors coupled to G_q activate this calcium overload. Finally, various studies have shown that participants in the G_q pathway, specifically IP_3 and its receptor, are important in the generation of arrhythmogenic calcium activity.

TRP CHANNELS

TRP channels are the last step in the store operated calcium entry pathway. The TRP gene encodes a superfamily of channels that contain 6 transmembrane helices with a pore-forming loop between the fifth and sixth helix and a cytosolic N and C terminus.[17] Each channel in this superfamily also contains a conserved TRP domain on the C terminus.[18] There are 7 subfamilies in the TRP superfamily: TRP Canonical (TRPC), TRP Vanilloid (TRPV), TRP Melastatin (TRPM), TRP Ankyrin (TRPA), TRP Mucolipin (TRPML), TRP Polycystin (TRPP), and No Mechanoreceptor Potential C (TRPN).[19,20]

TRPC CHANNELS

One family of TRP channels present in cardiac myocytes is the TRPC family. The TRPC family contains seven isoforms (TRPC1 to 7) expressed in mammalian cells that are further subdivided into 4 groups. The first group contains the isoforms TRPC4 and TRPC5. Both are highly selective for calcium, and are activated by calcium store depletion.[17] The second group consists solely of TRPC 1. This isoform is also operated by calcium store depletion, but is distinguished from the first group by forming a less selective calcium channel.[21] The third group consists of TRPC3, TRPC6, and TRPC7. These 3 are all non-selective cation channels activated by DAG, a store-independent mechanism,[22] though human TRPC3 has been shown to complex with TRPC1 to form a channel activated by a store-dependent mechanism.[23] TRPC2 forms the last group and is believed to be a pseudogene in humans.[19]

Of all the TRPC channels, several studies show that TRPC3 may be associated with various players involved in calcium signaling during excitation-contraction coupling. In cardiac muscle, the L-type calcium channel allows calcium to enter the cell and stimulate the ryanodine receptors during excitation-contraction cycling to release calcium from the sarcoplasmic reticulum, resulting in contraction. Kiselyov et al was able to show via co-immunopreciptation that TRPC3 directly interacted with both IP_3 receptors and ryanodine receptors. However, they could not show that ryanodine receptor 2, which is present in cardiac myocytes, couples to TRPC3.[24] This is similar to L-type calcium channels in cardiac myocytes. The channels do not directly couple, but still regulate ryanodine receptor 2. They hypothesized that calcium release from the sarcoplasmic reticulum through ryanodine receptors may stimulate calcium entry through TRPC3.[24] In addition to IP_3 receptor calcium release, this activation mechanism may provide a more robust signal to TRPC3, since ryanodine receptors outnumber IP_3 receptors in the cardiac myocyte.

As the previous study indicated, TRPC3 interacted with players of normal excitation-contraction coupling; these studies suggest that it does so under pathophysiological conditions. After contraction in normal excitation-contraction cycling, the sodium calcium exchanger transports calcium out of the cell while sodium is brought into the cell, resulting in myocyte relaxation. During pathophysiological conditions, the sodium calcium exchanger can also work in reverse and bring calcium into the cell. Since TRPC3 is a non-selective cation channel and has a significant sodium permeability, Eder et al hypothesize that sodium entry through TRPC3 activates the reverse mode of the sodium calcium exchanger, resulting in increased intracellular calcium concentration. They were able to co-immunoprecipitate TRPC3 and the sodium calcium exchanger in the membrane fraction of rat ventricular myocytes. In their study, they also showed that angiotensin II, an activator of the Gq pathway, greatly augmented the increase in intracellular calcium attributed to the sodium calcium exchanger operating in reverse mode. This increase was diminished when the myocytes were transiently transfected with an N-terminal fragment of TRPC3, which inhibits the channel.[25] A study of mouse myocytes from the sinoatrial node (the pacemaker center of the right atria) found that that the inhibitor of the reverse mode of the sodium calcium exchanger, KBR-7943, only inhibited store operated calcium entry by 25%. In addition, the L-type calcium channel blocker, nifedipine, had no effect on store-operated calcium entry.[26] These two studies could represent the different signaling pathways present in pacemaker cells when compared to other cardiac myocytes. Taken together, they show a relationship between TRPC3 and store operated calcium entry in the heart. More studies are needed to determine the extent of the reverse mode sodium calcium exchanger's involvement in store operated calcium entry in various cell types.

TRPM CHANNELS

Another family of TRP channels present in cardiac myocytes is the TRPM family. There are 8 isoforms in the TRPM family (TRPM1 to 8) expressed in mammalian cells that are further subdivided into 4 groups. TRPM1 and TRPM3 make up the first group and have high calcium permeability. While TRPM1 may be spontaneously active, TRPM3 is voltage dependent. The second group is comprised of TRPM4 and TRPM5. Both are calcium activated monovalent-permeable, non-selective cation channels. The third group consists of TRPM6 and TRPM7 channels that are permeable to divalent ions such as calcium. Studies show TRPM7 to be regulated by cAMP levels. TRPM2 and TRPM8 are also permeable to calcium and make up the final group of TRPM channels. While TRPM2 is activated by ADP-ribose levels, TRPM8 is activated by cell cooling.[27]

One lab has conducted several studies on TRPM channels and their relationship with AF. Guinamard and associates describe non-selective cation channels as one component of the calcium dependent transient inward current believed to be involved in generating arrythmogenic activity such as delayed after-depolarizations. In isolated human atrial myocytes, they used patch-clamp techniques to determine the characteristics of the non-selective cation channel. They found that TRPM4b and TRPM5 both share the properties of this non-selective cation channel. Specifically, they found TRPM4b mRNA expression in the isolated human atrial myocytes.[28] The same group later determined that TRPM4 is the candidate for the non-selective cation channel current responsible for delayed after-depolarizations in mouse sino-atrial node pacemaker cells. They found that the open probability of the channel increased with the addition of PIP_2 to the inner side of the membrane. [29] Though they did not associate TRPM4 activation to store operated calcium entry, they did show that activation of the channel involves components of the G_q signaling pathway. In addition, they directly correlated TPRM4 with pathophysiological calcium signaling activity that may be involved in AF.

ATRIAL FIBRILLATION AND CONGESTIVE HEART FAILURE

One of the most common serious complication that occurs with AF is congestive heart failure (CHF).[30] CHF is a condition in which the heart's ability to deliver oxygen-rich blood is inadequate to meet the body's needs. The presence of CHF alone increases the risk of developing AF,[31-34] and those who have AF and CHF concurrently have a worse prognosis than those with CHF alone.[35] As the heart overfills with blood during CHF, the atria undergo chronic atrial dilatation or stretch. Chronic stretch causes atrial hypertrophy and the development of interstitial fibrosis; both contribute to the development of AF.[36] In addition, chronic stretch also causes electrophysiological changes in atrial myocytes that result in alterations in calcium handling.[37,38]

G_q AND TRP SIGNALING IN CONGESTIVE HEART FAILURE

As in AF, G_q appears to be important in the hypertrophic response to heart failure. In the ventricular myocardium of dogs, G_q expression increased in failing hearts as compared to normal hearts.[39] In fact, expression of a cardiomyocyte-specific $G_{q/11}$ deficient gene was found to reduce the hypertrophic response of the pressure overloaded heart.[40] Another study showed that in patients with mitral valve disease, PLC activation increases as atrial size increases.[41] These studies indicate that the G_q signaling pathway plays a considerable role in the development of hypertrophy seen during CHF.

During CHF, stretch activated channels in the hypertrophic heart are thought to partially mediate the downstream electrophysiological changes initiated by AF.[42,43] In rabbit hearts, Bode et al were able to inhibit the induction of AF using gadolinium, a stretch-activated current blocker.[42] The identity of the stretch activated channels is unknown. However, deregulation of calcium signaling was found to contribute to the production of arrhythmogenic substrate during CHF.[30,44] Maroto et al used frog oocyte membranes containing the stretch activated channel present in vertebrates. They found that they could inhibit the activity of this channel using TRPC1-specific antisense RNA.[45] On the other hand, using human embryonic kidney 293 cells, another group proposed TRPC6 is a candidate for the stretch activated channel.[46] Even though TRPC6 is not a classical stretch activated channel, membrane stretch has been shown to activate the G_q coupled receptor for angiotensin II (the AT1 receptor) in an agonist independent manner.[47,48] These studies need to be done in atrial myocytes before conclusions can be made as to which TRPC channel is responsible for the stretch activated current associated with CHF and AF.

TRPC INDUCED HYPERTROPHIC GENE EXPRESSION IN CONGESTIVE HEART FAILURE

In the CHF setting, calcium activates certain transcription factors to transcribe the hypertrophy gene program. One of these transcription factors, NFAT, is activated by a sustained increase in calcium concentration. During EC coupling, the calcium concentration is increased dramatically by calcium entry through voltage dependent L type calcium channels, stimulating a substantial release of calcium from the sarcoplasmic reticulum through the ryanodine recpetors. Since this increase in calcium is reversible within milliseconds, 1 study hypothesized that a candidate for the sustained increase in calcium necessary for NFAT activation is TRPC1. They studied abdominal aortic banded rats to determine the role of store operated calcium entry through TRPC1 in hypertrophy. Of the 4 TRPC isoforms that were detected, only TRPC1 was elevated in hearts of these rats when compared to sham surgery rats. In the same study, they silenced the TRPC1 gene using small interfering RNA and found that endothelin I induced store operated calcium entry was inhibited in primary cultures of cardiomyocytes from neonatal rats. This indicates that TRPC1 may play a large role in store operated calcium entry responsible for activation of NFAT and the hypertrophic gene program.[49]

Other studies of neonatal rats also found TRPC3 to be the major player in activation of the hypertrophic gene program. One such study found that TRPC3 was the only TRPC isoform to have increased mRNA levels in response to 60-hour phenylephrine treatment resulting in hypertrophy. This lab also found that infecting these cells with short hairpin RNA targeting TRPC3 and reducing TRPC3 expression reduced the baseline and the amplitude of the phenylephrine induced calcium transient.[50] A second study showed TRPC3 and TRPC6 activation via DAG, a store independent mechanism, is required for NFAT activation induced by hypertrophy and angiotensin II.[51]

All of the aforementioned studies done on TRPC induced hypertrophic gene expression were conducted in neonatal rats. The TRPC1 study was performed in myocytes isolated from the whole heart, whereas the TRPC3 and TRPC6 studies only used myocytes from the ventricle. It is difficult to draw conclusions about the signaling events in the atria based on studies done in the ventricle. In the rat, Lipp et al. found atrial myocytes had approximately 6 times the expression of one of the most abundant IP_3 receptor isoforms (type II) than did ventricular myocytes.[52] Therefore, these studies could indicate differences in signaling pathways between the ventricle and atrium, or that both TRPC1 and TRPC3 form a heteromeric channel to activate hypertrophic gene expression.

CONCLUSIONS

Abnormal calcium signaling plays a critical role in the development and continuation of AF. Current pharmacologic therapies target calcium signaling of the normal excitation-contraction coupling pathway. A better approach may be to target the calcium signaling that occurs during pathophysiological conditions. The various studies mentioned demonstrate a role for G_q mediated store operated calcium entry and TRP channels in AF as well as the concurrent hypertrophy and CHF that may occur. Targeting this pathway and these channels may provide a possible treatment for these conditions that do not interfere with normal excitation-contraction coupling.

References

1. Centers for Disease Control and Prevention. Atrial fibrillation fact sheet. http://www.cdc.gov/dhdsp/library/pdfs/fs_atrial_fibrillation.pdf. Published February 2010. Accessed October 5, 2010.

2. Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation.* 2010;121(7):e46-e215.

3. Van Wagoner DR, Pond AL, Lamorgese M, Rossie SS, McCarthy PM, Nerbonne JM. Atrial L-type Ca^{2+} currents and human atrial fibrillation. *Circ Res.* 1999;85(5):428-436.

4. Hove-Madsen L, Llach A, Bayes-Genis A, et al. Atrial fibrillation is associated with increased spontaneous calcium release from the sarcoplasmic reticulum in human atrial myocytes. *Circulation.* 2004;110(11):1358-1363.

5. Goette A, Honeycutt C, Langberg JJ. Electrical remodeling in atrial fibrillation: time course and mechanisms. *Circulation.* 1996;94(11):2968-2974.

6. Wang C, Jayadev S, Escobedo JA. Identification of a domain in the angiotensin II type 1 receptor determining G coupling by the use of receptor chimeras. *J Biol Chem.* 1995;270(28):16677-16682.

7. Sokolovsky M. Functional coupling between endothelin receptors and multiple G-proteins in rat heart myocytes. *Receptors Channels.* 1993;1(4):295-304.

8. LaMorte VJ, Thorburn J, Absher D, et al. Gq- and ras-dependent pathways mediate hypertrophy of neonatal rat ventricular myocytes following alpha 1-adrenergic stimulation. *J Biol Chem.* 1994;269(18):13490-13496.

9. Gassanov N, Brandt MC, Michels G, Lindner M, Er F, Hoppe UC. Angiotensin II-induced changes of calcium sparks and ionic currents in human atrial myocytes: potential role for early remodeling in atrial fibrillation. *Cell Calcium.* 2006;39(2):175-186.

10. Schnabel P, Gäs H, Nohr T, Camps M, Böhm M. Identification and characterization of G protein-regulated phospholipase C in human myocardium. *J Mol Cell Cardiol.* 1996;28(12):2419-2427.

11. Zima AV, Blatter LA. Inositol-1,4,5-trisphosphate-dependent Ca^{2+} signalling in cat atrial excitation-contraction coupling and arrhythmias. *J Physiol.* 2004;555(3):607-615.

12. Randriamampita C, Tsien RY. Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. *Nature.* 1993;364(6440):809-814.

13. Mackenzie L, Bootman MD, Laine M, et al. The role of inositol 1,4,5-trisphosphate receptors in Ca^{2+} signalling and the generation of arrhythmias in rat atrial myocytes. *J Physiol.* 2002;541(2):395-409.

14. Li X, Zima AV, Sheikh F, Blatter LA, Chen J. Endothelin-1-Induced arrhythmogenic Ca^{2+} signaling is abolished in atrial myocytes of inositol-1,4,5-trisphosphate (IP_3)-receptor type 2-deficient mice. *Circ Res.* 2005;96(12):1274-1281.

15. Kaplan P, Jurkovicova D, Babusikova E, et al. Effect of aging on the expression of intracellular Ca^{2+} transport proteins in a rat heart. *Mol Cell Biochem.* 2007;301(1):219-226.

16. Liang X, Xie H, Zhu PH, et al. Enhanced activity of inositol-1,4,5-trisphosphate receptors in atrial myocytes of atrial fibrillation patients. *Cardiology.* 2009;114(3):180-191.

17. Nilius B, Droogmans G. Ion channels and their functional role in vascular endothelium. *Physiol Rev.* 2001;81(4):1415-1459.

18. Nilius B, Droogmans G, Wondergem R. Transient receptor potential channels in endothelium: solving the calcium entry puzzle? *Endothelium.* 2003;10(1):5-15.

19. Montell C, Birnbaumer L, Flockerzi V. The TRP Channels, a remarkably functional family. *Cell.* 2002;108(5):595-598.

20. Pedersen SF, Owsianik G, Nilius B. TRP channels: an overview. *Cell Calcium.* 2005;38(3-4):233-252.

21. Liu X, Wang W, Singh BB, et al. Trp1, a candidate protein for the store-operated Ca^{2+} influx mechanism in salivary gland cells. *J Biol Chem.* 2000;275(5):3403-3411.

22. Dietrich A, Mederos y Schnitzler M, Emmel J, Kalwa H, Hofmann T, Gudermann T. N-linked protein glycosylation is a major determinant for basal TRPC3 and TRPC6 channel activity. *J Biol Chem.* 2003;278(48):47842-47852.

23. Liu X, Bandyopadhyay BC, Singh BB, Groschner K, Ambudkar IS. Molecular analysis of a store-operated and 2-acetyl-sn-glycerol-sensitive non-selective cation channel. *J Biol Chem.* 2005;280(22):21600-21606.

24. Kiselyov KI, Shin DM, Wang Y, Pessah IN, Allen PD, Muallem S. Gating of store-operated channels by conformational coupling to ryanodine receptors. *Mol Cell.* 2000;6(2):421-431.

25. Eder P, Probst D, Rosker C, et al. Phospholipase C-dependent control of cardiac calcium homeostasis involves a TRPC3-NCX1 signaling complex. *Cardiovasc Res.* 2007;73(1):111-119.

26. Ju Y-K, Chu Y, Chaulet H, et al. Store-operated Ca^{2+} influx and expression of trpc genes in mouse sinoatrial node. *Circ Res.* 2007;100(11):1605-1614.

27. Kraft R, Harteneck C. The mammalian melastatin-related transient receptor potential cation channels: an overview. *Pflugers Arch.* 2005;451(1):204-211.

28. Guinamard R, Chatelier Al, Demion M, et al. Functional characterization of a Ca^{2+}-activated non-selective cation channel in human atrial cardiomyocytes. *J Physiol.* 2004;558(1):75-83.

29. Demion M, Bois P, Launay P, Guinamard R. TRPM4, a Ca^{2+}-activated nonselective cation channel in mouse sino-atrial node cells. *Cardiovasc Res.* 2007;73(3):531-538.

30. Heist EK, Ruskin JN. Atrial fibrillation and congestive heart failure: risk factors, mechanisms, and treatment. *Prog Cardiovasc Dis.* 2006;48(4):256-269.

31. Li D, Fareh S, Leung TK, Nattel S. Promotion of atrial fibrillation by heart failure in dogs : atrial remodeling of a different sort. *Circulation.* 1999;100(1):87-95.

32. Van den Berg MP, Tuinenburg AE, Crijns HJ, Van Gelder IC, Gosselink AT, Lie KI. Heart failure and atrial fibrillation: current concepts and controversies. *Heart.* 1997;77:309-313.

33. Spach MS. Mounting evidence that fibrosis generates a major mechanism for atrial fibrillation. *Circ Res.* 2007;101(8):743-745.

34. Sridhar A, Nishijima Y, Terentyev D, et al. Chronic heart failure and the substrate for atrial fibrillation. *Cardiovasc Res.* 2009;84(2):227-236.

35. Maisel WH, Stevenson LW. Atrial fibrillation in heart failure: epidemiology, pathophysiology, and rationale for therapy. *Am J Cardiol.* 2003;91(6)(suppl):2-8.

36. Ben Morrison T, Jared Bunch T, Gersh BJ. Pathophysiology of concomitant atrial fibrillation and heart failure: implications for management. *Nat Clin Pract Cardiovasc Med.* 2009;6(1):46-56.

37. Chorro FJ, Trapero I, Such-Miquel L, et al. Pharmacological modifications of the stretch-induced effects on ventricular fibrillation in perfused rabbit hearts. *Am J Physiol Heart Circ Physiol.* 2009;297(5):H1860-H1869.

38. Sanders P, Morton JB, Davidson NC, et al. Electrical remodeling of the atria in congestive heart failure: electrophysiological and electroanatomic mapping in humans. *Circulation.* 2003;108(12):1461-1468.

39. Suematsu N, Satoh S, Kinugawa S, et al. {alpha}1-Adrenoceptor-G_q-RhoA signaling is upregulated to increase myofibrillar Ca^{2+} sensitivity in failing hearts. *Am J Physiol Heart Circ Physiol.* 2001;281(2):H637-H646.

40. Wettschureck N, Rutten H, Zywietz A, et al. Absence of pressure overload induced myocardial hypertrophy after conditional inactivation of G[alpha]q/G[alpha]11 in cardiomyocytes. *Nat Med.* 2001;7(11):1236-1240.

41. Woodcock EA, Grubb DR, Filtz TM, et al. Selective activation of the "b" splice variant of phospholipase C[beta]1 in chronically dilated human and mouse atria. *J Mol Cell Cardiol.* 2009;47(5):676-683.

42. Bode F, Katchman A, Woosley RL, Franz MR. Gadolinium decreases stretch-induced vulnerability to atrial fibrillation. *Circulation.* 2000;101(18):2200-2205.

43. Stiber JAMD, Seth MP, Rosenberg PBMD. Mechanosensitive channels in striated muscle and the cardiovascular system: not quite a stretch anymore. *J Cardiovasc Pharmacol.* 2009;54(2):116-122.

44. Yeh Y-H, Wakili R, Qi X-Y, et al. Calcium-handling abnormalities underlying atrial arrhythmogenesis and contractile dysfunction in dogs with congestive heart failure. *Circ Arrhythm Electrophysiol.* 2008;1(2):93-102.

45. Maroto R, Raso A, Wood TG, Kurosky A, Martinac B, Hamill OP. TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat Cell Biol.* 2005;7(2):179-185.

46. Spassova MA, Hewavitharana T, Xu W, Soboloff J, Gill DL. A common mechanism underlies stretch activation and receptor activation of TRPC6 channels. *Proc Natl Acad Sci U S A.* 2006;103(44):16586-16591.

47. Dietrich A, Chubanov V, Kalwa H, Rost BR, Gudermann T. Cation channels of the transient receptor potential superfamily: their role in physiological and pathophysiological processes of smooth muscle cells. *Pharmacol Ther.* 2006;112(3):744-760.

48. Dietrich A, Kalwa H, Storch U, et al. Pressure-induced and store-operated cation influx in vascular smooth muscle cells is independent of TRPC1. *Pflugers Arch.* 2007;455(3):465-477.

49. Ohba T, Watanabe H, Murakami M, et al. Upregulation of TRPC1 in the development of cardiac hypertrophy. *J Mol Cell Cardiol.* 2007;42(3):498-507.

50. Brenner JS, Dolmetsch RE. TrpC3 regulates hypertrophy-associated gene expression without affecting myocyte beating or cell size. *PLoS One.* 2007;2(8):e802.

51. Onohara N, Nishida M, Inoue R, et al. TRPC3 and TRPC6 are essential for angiotensin II-induced cardiac hypertrophy. *EMBO J.* 2006;25(22):5305-5316.

52. Lipp P, Laine M, Tovey SC, et al. Functional $InsP_3$ receptors that may modulate excitation-contraction coupling in the heart. *Curr Biol.* 2000;10(15)(suppl):939-942.