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Ca²⁺ Entry Through TRP-C Channels Regulates Fibroblast Biology in Chronic Atrial Fibrillation

Robert A. Rose, PhD; Darrell D. Belke, PhD; Mary M. Maleckar, PhD; Wayne R. Giles, PhD

In this issue of *Circulation*, Harada et al¹ provide fundamental new insights into the cellular mechanism(s) for initiation and maintenance of chronic atrial fibrillation in the human heart. The authors, taking what most might still consider to be an unconventional approach to understanding this proarrhythmic substrate,^{2,3,4} have identified the atrial fibroblast as an important player. More specifically, this international group of investigators concludes that a particular member of the transient receptor potential or TRP family⁵ of ion channels, TRPC3, when expressed/upregulated in human atrial fibroblasts, can contribute to chronic atrial fibrillation. Activation or enhanced expression of TRPC3 provides a means for increased transmembrane calcium entry into the fibroblast. This trigger calcium can then result in a marked increase in proliferation, followed by transformation to the myofibroblast phenotype.^{6,7} A previous study had drawn attention to the possibility that a different TRP channel subtype, TRPM7, could play a somewhat similar proarrhythmic role in the atrium.^{7,8}

Article see p 2051

Atrial fibrillation is the most common form of cardiac arrhythmia in adult humans.⁹ Importantly, its incidence is projected to increase substantially as a consequence of the association of atrial fibrillation with healthy aging,¹⁰ diabetes mellitus, and hypothyroidism.

Harada et al¹ provide the first evidence for the presence of TRPC3 current during the proliferative phase in cultured human atrial fibroblasts. Knockdown of TRPC3 is able to suppress atrial fibroblast proliferation, and similar results were obtained with the pharmacological inhibitor PYR3, a relatively new pyrazole-based compound. This particular TRP channel exhibits significant calcium permeability. This calcium influx contributes to ERK phosphorylation, which is involved in mediating atrial fibroblast proliferation. A very interesting observation is that TRPC expression is markedly reduced and can even disappear when the proliferating fibroblasts eventually adopt a myofibroblast phenotype.

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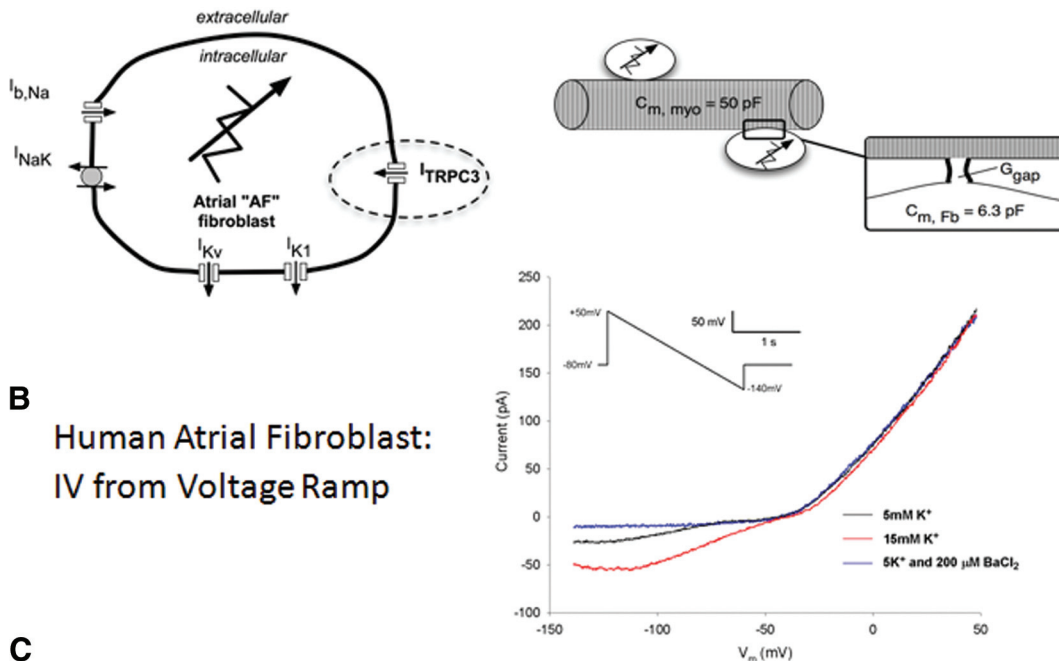
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Previously, we have shown that members of the TRPC family are importantly involved in the electrophysiological mechanisms that underlie some of the effects of natriuretic peptides (acting through the natriuretic peptide C receptor) in rat ventricle.⁸ In this case (although calcium entry and subsequent intracellular calcium waves and altered gene transcription are likely consequences), the electrogenic current through TRP channels is sufficient that connexin-mediated electrotonic cell–cell communication from the fibroblast or myofibroblast to the myocyte is altered. This intercellular current flow can change the resting potential, the action potential waveform, or both.

We have explored some of these TRP channel–mediated effects using mathematical models of the human atrial myocyte in the presence or absence of electrotonic interaction with a selected number of fibroblasts.^{10,11} This *in silico* approach can provide useful insights into the consequences of data sets such as those in the Harada et al¹ study. Panel A of the Figure consists of 2 schematics that show the chosen model components. Panel B is a current–voltage (I–V) relationship. It shows the major background potassium current and voltage activated potassium current. These two conductances produce changes in resting membrane potential and promote electrotonic repolarization in the human atrial fibroblast.¹² Panel C of the Figure combines this information. It is based on the results of a computation done using 1 human myocyte coupled with 1 fibroblast through an intercellular resistance that is similar to that which has been measured experimentally (c.f. refs 10, 11). The calculated depolarization of the resting potential draws attention to the possibility that activation of a TRPC3 conductance in the fibroblast when scaled to match the current density in Harada et al¹ may have a significant influence on the resting potential of atrial myocytes. Even a small depolarization in the atrium would reduce excitability and thus could alter the atrial tissue substrate even if only 1 fibroblast (on average) were coupled to each myocyte, as this computation assumes.

In the section of this article¹ that addresses the molecular mechanisms that regulate TRPC3 expression during the development of atrial fibrillation, an important role for the microRNA species, denoted 26a/b, is identified. The motivation for this set of experiments is based in part on previous work that demonstrated strong modulation of cardiac fibrosis by selected microRNA entities.¹³ Specifically, MicroRNA-26 is shown to negatively regulate the expression of TRPC3 as judged by results from gain- and loss-of-function experiments. Furthermore, an important role for NFATc3 (but not NFATc4) that is localized to the nucleus in mediating the repression of miRNA-26 is identified. In summary, the intracellular signaling data provided by Hirada et al¹ demon-

A Proposed coupling between myocytes and fibroblasts



B Human Atrial Fibroblast: IV from Voltage Ramp

C Simulated Action Potential from a Human Atrial Myocyte

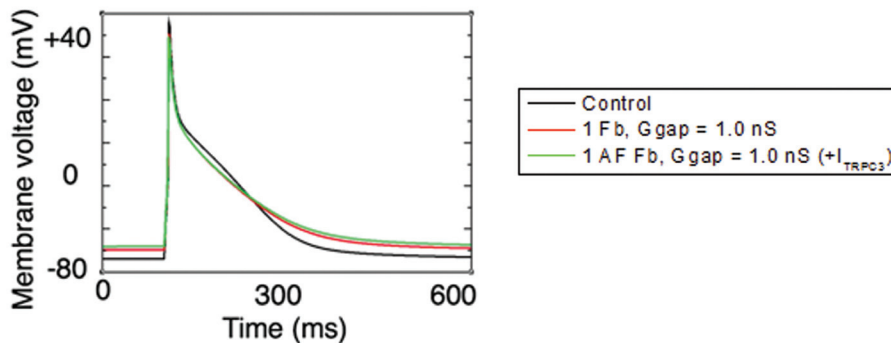


Figure. Possible electrophysiological consequences of TRPC3 current activation in human atrium. **A (left)**, Schematic diagram of an atrial fibroblast with known ionic currents designated, including a TRPC3 conductance. **A (right)**, Connexin-mediated myocyte to fibroblast cell-cell coupling. **B**, Current-voltage data that are representative of the potassium currents in human atrial fibroblasts recorded using perforated patch methods.^{11,12} The 3 superimposed current-voltage curves illustrate the approximate current density of the major potassium currents in this fibroblast preparation from the human right atrial appendage. The significant decrease in inward current after BaCl₂ (200 μmol/L) application suggests the presence of an inward rectifying background potassium current. **C**, Three superimposed action potentials computed at a stimulus frequency of 1 Hz based on our mathematical model of the human atrial action potential.¹³ The black trace is the control record from an isolated human atrial myocyte in silico. The red action potential waveform was obtained after coupling of 1 fibroblast expressing background potassium conductances similar to those shown in **B**. The green trace was obtained under conditions identical to the red trace except that a quasi linear conductance scaled to match the TRPC-3 data in Figure 1B and 4B of Harada et al¹ was added. See text for further description and explanation.

strate that downregulation of miRNA-26 results in selective increase in the expression of TRPC3, and this is followed by enhanced fibroblast proliferation.

The finding that NFATc3 is involved in mediating fibroblast proliferation is interesting and potentially very important. It is now known that this transcription factor can mediate ventricular hypertrophy. The hypertrophic phenotype includes fibroblast proliferation and altered conduction patterns.¹⁴ The translocation of NFATc3 within the nucleus, which apparently is essential for fibroblast proliferation, has recently been shown to be dependent on protein O-GlcNAc modification.¹⁵ Interestingly, increased propensity for protein O-GlcNAcylation is a hallmark of diabetes mellitus.¹⁶ This

biochemical modulation has also been demonstrated to occur in the heart as a result of aging. Diabetes mellitus and the aging process are both associated with an increased incidence of atrial rhythm disturbances, including atrial fibrillation.⁹

In summary, Harada et al¹ have provided a fundamental advance in understanding the cell physiology and intracellular signaling mechanisms involved in fibrosis during atrial fibrillation. Their findings draw attention to a strong link between fibroblast ion channels and the proarrhythmic substrate in the atrium. As presented, these findings suggest that calcium entry through the TRPC3 channels is critically important for triggering calcium-dependent intracellular signaling mechanisms that can regulate fibroblast proliferation

and perhaps also modulate the transition to the myofibroblast phenotype. However, these results need to be considered in the context of quite complex calcium homeostasis in both the normal and the compromised human atrial myocyte (see Grandi et al¹⁷).

A recent study that reveals the expression of cardiac sodium channels in myofibroblasts from human atrium¹⁸ also provides an important perspective when the clinical significance of the findings in Harada et al¹ are being considered. If the myocyte-fibroblast-myofibroblast syncytium that contributes to the characteristic substrate found in chronic atrial fibrillation includes the possibility that the myofibroblast could support a sodium current-mediated regenerative response, virtually all present concepts for pharmacological management of atrial rhythm disturbances need to be reconsidered.^{10,17,19} The possibility that transformed fibroblasts could correspond to excitable tissue also needs to be considered when so-called biological therapies for cardiac rhythm disturbances are being refined and further developed.²⁰ This opportunity and significant challenge also needs to be factored into the rapidly emerging field that focuses on heart repair by reprogramming fibroblasts. This approach is based on a cocktail of transcription factors being introduced into uniform, sometimes autologous, populations of cardiac tissue-specific fibroblasts to bring about repair which are then placed in the myocardium.^{21–23}

Disclosures

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KEY WORDS: Editorials ■ atrial fibrillation ■ calcium ■ cardiac fibroblast ■ TRP channels