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Mechanisms of spontaneous Ca2+ release-mediated arrhythmia in a novel 3D human atrial myocyte model: II. Ca2+-handling protein variation

Xianwei Zhang, Charlotte Smith, Stefano Morotti, Andrew Edwards, Daisuke Sato, William E. Louch, Haibo Ni, and Eleonora Grandi
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1st Editorial Decision **12-Aug-2022**

Dear Professor Grandi,

Re: JP-RP-2022-283602 "Mechanisms of spontaneous Ca2+ release-mediated arrhythmia in a novel 3D human atrial myocyte model: II. Ca2+-handling protein variation" by Xianwei Zhang, Charlotte Smith, Stefano Morotti, Andrew Edwards, Daisuke Sato, William E. Louch, Haibo Ni, and Eleonora Grandi

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert Referees and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

Please advise your co-authors of this decision as soon as possible.

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If you have any queries please reply to this email and staff will be happy to assist.

Yours sincerely,

Bjorn Knollmann
Senior Editor
The Journal of Physiology

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paper should also be publicly archived. If sharing data compromises ethical standards or legal requirements then authors are not expected to share it, but must note this in their Statement. For more information, see our Statistics Policy.

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EDITOR COMMENTS

Reviewing Editor:

Both reviewers commented on the quality of the work and it's likely impact. Subject to some minor revisions as requested, both papers should be acceptable as back-to-back publications. Congratulations on some very nice work.

REFEREE COMMENTS

Referee #1:

This work presents new information regarding the contribution of Ca handling proteins in atrial myocytes using computer model. The authors developed and validated a new model of 3D human atrial myocyte in the companion paper. Here, they examined the impact of TATS density and changes in expression and distribution of Ca-handling proteins (Na-Ca exchanger, Ryanodine receptor, Calsquestrin) related to atrial fibrillation (10 to 200% changes). For all 3 key actors, varying expression and localization has pro and anti-arrhythmic effects (investigated as spontaneous Ca release, delayed after depolarization and spontaneous action potential). Biphasic effects are observed. Intermediately tubulated atrial myocytes showed profound effect whereas detubulated (sparse) myocytes appear unaffected and densely tubulated protected. Inner uncoupled Ca release units appear the main culprit for arrhythmia. Interestingly some modeling data showed change in Cai-Vm coupling.

This study is a logical follow-up of the companion paper. The model data are compared to relevant literature (cardiac disease, KO mice) and go even further. We can clearly see the power of computer modeling. As the previous paper, this is an excellent study and will benefit the field. The study is well-written and well executed. I have very few concerns to raise, mostly some suggestions for the authors to expand upon:

In this paper atrial fibrillation is well explained, but see my comments on the first study, where it should be introduced as well and therefore re-arrange the 2 papers.

Two translational points could be more discussed:

The modeling data suggest that increasing Na-Ca exchanger density may be protective against Ca overload. As the authors wrote currently an inhibition is though to more relevant clinically. A special issue in Cell Calcium deals with "Na-dependent transporters" not only in the heart and Na-Ca exchanger activators are presented in neurons (Cell Calcium, 2020 vol 87). Can the authors discuss briefly this point?

The modeling data suggest that intermediately tubulated atrial myocytes are more prone to arrhythmogenic events. One way to reduce these events would be to "re-tubulate" cells and this is also a great deal in heart failure for ventricular myocytes. Recently, several studies are interested in the formation/proliferation of t-tubules not only in myocytes but also in iPS, a small transitional paragraph about this point could be add.

Referee #2:

This paper assesses the impact TATS remodeling along with variying the subcellular distribution of NCX1, RyR2, and CSQ to atrial arrhythmogenesis with the use of a new computational model. The authors show that the variation of Ca handling proteins had the strongest impact in cells with intermediate TATS. This paper is a nice extension of the accompanying paper, in which the computational model was built. My comments are only minor.

- Again SCRs and DADs occur in isolated atrial myocytes from sinus rhythm and AF patients (more in the later than in the former) in the absence of t-tubule and TATS. I suggest to stress this issue to the readers and add some discussion on this issue.

- RyR2 variation: SCRs are increased in patients with paroxysmal AF (Beavers et al., JACC 2013; Voigt et al., Circ 2014) and this occurs likely because of increased RyR2 channel expression in the face of no t-tubules and TATS. You should also refer to the many animal models of atria tachycardia remodeling in which a reduction of total RyR2 expression also occur as part of the underlying proarrhythmic substrate.

- RyR2 function is also increased in the atria of patients with HFrEF, despite the fact that total RyR2 levels were reduced (PMID: 30356673). This should mentioned.

- LCC variation: Christ et al. 2004 do not show a decrease in LCC expression, although the current is reduced in AF patients.

END OF COMMENTS

Confidential Review **18-Jul-2022**

Responses to Reviewers **Zhang et al. JP-RP-2022-283602R1**

EDITOR COMMENTS

Reviewing Editor:
Both reviewers commented on the quality of the work and it's likely impact. Subject to some minor revisions as requested, both papers should be acceptable as back-to-back publications. Congratulations on some very nice work.

Senior Editor:
I concur with the expert reviewing editor's assessment. Excellent work.

We thank the reviewing and senior editors for their positive notes. We appreciate the reviewer's assessment and critiques and have addressed all comments in the revised manuscript. Specific changes are highlighted in our point-by-point rebuttal and tracked in the manuscript.

Referee #1:

This work presents new information regarding the contribution of Ca handling proteins in atrial myocytes using computer model. The authors developed and validated a new model of 3D human atrial myocyte in the companion paper. Here, they examined the impact of TATS density and changes in expression and distribution of Ca-handling proteins (Na-Ca exchanger, Ryanodine receptor, Calsquestrin) related to atrial fibrillation (10 to 200% changes). For all 3 key actors, varying expression and localization has pro and anti-arrhythmic effects (investigated as spontaneous Ca release, delayed after depolarization and spontaneous action potential). Biphasic effects are observed. Intermediately tubulated atrial myocytes showed profound effect whereas detubulated (sparse) myocytes appear unaffected and densely tubulated protected. Inner uncoupled Ca release units appear the main culprit for arrhythmia. Interestingly some modeling data showed change in Cai-Vm coupling.

This study is a logical follow-up of the companion paper. The model data are compared to relevant literature (cardiac disease, KO mice) and go even further. We can clearly see the power of computer modeling. As the previous paper, this is an excellent study and will benefit the field. The study is well-written and well executed. I have very few concerns to raise, mostly some suggestions for the authors to expand upon:
We appreciate the reviewer's positive assessment and useful suggestions for improvement.

1. In this paper atrial fibrillation is well explained, but see my comments on the first study, where it should be introduced as well and therefore re-arrange the 2 papers.
Thank you, point well taken. We chose to add a paragraph in the first paper rather than rearranging content.

Two translational points could be more discussed:

2. The modeling data suggest that increasing Na-Ca exchanger density may be protective against Ca overload. As the authors wrote currently an inhibition is though to more relevant clinically. A special issue in Cell Calcium deals with "Na-dependent transporters" not only in the heart and

Na-Ca exchanger activators are presented in neurons (Cell Calcium, 2020 vol 87). Can the authors discuss briefly this point?
Thank you for this suggestion. We added a brief discussion on Na-Ca exchanger activators (lines 466-474): ***"While NCX inhibition is thought to be more relevant to cardiac disease clinically, it is interesting to note that pharmacological NCX activators are emerging as promising strategies to ameliorate certain neurodegenerative diseases (Annunziato et al., 2020), such as stroke, neonatal hypoxia, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and spinal muscular atrophy. In neuronal cells, various NCX isoforms work to maintain Na^+ and Ca^{2+} homeostasis in the cytosol, endoplasmic reticulum, and mitochondria via both forward and reverse mode operation; and have been shown to increase survival of neuronal and glial-derived cells in pathophysiologic conditions (Annunziato et al., 2020; Pannaccione et al., 2020)."***

3. The modeling data suggest that intermediately tubulated atrial myocytes are more prone to arrhythmogenic events. One way to reduce these events would be to "re-tubulate" cells and this is also a great deal in heart failure for ventricular myocytes. Recently, several studies are interested in the formation/proliferation of t-tubules not only in myocytes but also in iPS, a small transitional paragraph about this point could be add.
We agree and briefly discussed current efforts to "re-tubulate" adult myocytes and iPS (lines 413-418): ***"Recent studies have investigated t-tubule restoration as a therapeutic maneuver in cardiac disease, especially in HF, and suggest that therapeutic t-tubule protection and repair may benefit inotropy while inhibiting arrhythmia (Manfra et al., 2017). Analogously, several groups endeavored to optimize experimental conditions to produce human induced pluripotent derived cardiomyocytes with functional t-tubule networks (Parikh et al., 2017)."***

Referee #2:

This paper assesses the impact TATS remodeling along with varying the subcellular distribution of NCX1, RyR2, and CSQ to atrial arrhythmogenesis with the use of a new computational model. The authors show that the variation of Ca handling proteins had the strongest impact in cells with intermediate TATS. This paper is a nice extension of the accompanying paper, in which the computational model was built. My comments are only minor.

1. Again SCRs and DADs occur in isolated atrial myocytes from sinus rhythm and AF patients (more in the later than in the former) in the absence of t-tubule and TATS. I suggest to stress this issue to the readers and add some discussion on this issue.
We agree and added this also in the introduction of this second paper, which indeed deals with studying other factors that may be contributing to the proarrhythmic behavior at various stages of TATS remodeling (lines 116-122): ***"Nevertheless, SCRs and DADs occur more frequently in AF vs. sinus rhythm human atrial myocytes that both mostly lack TATS after isolation via enzymatic digestion (though a fairly robust TATS presence is seen in human atrial tissue (Richards et al., 2011)). Indeed, the reduced density and regularity of the TATS is one aspect of disease remodeling, and it occurs concomitantly with altered channel and transporter expression, regulatory state, and function, as well as subcellular redistribution of ion channels, transporters, and Ca^{2+} handling proteins."***

2. RyR2 variation: SCRs are increased in patients with paroxysmal AF (Beavers et al., JACC 2013; Voigt et al., Circ 2014) and this occurs likely because of increased RyR2 channel

expression in the face of no t-tubules and TATS. You should also refer to the many animal models of atria tachycardia remodeling in which a reduction of total RyR2 expression also occur as part of the underlying proarrhythmic substrate.
We added these important papers (lines 494-498). "***SCRs are increased in myocytes isolated from patients with paroxysmal AF lacking TATS likely because of increased RyR channel expression (Beavers et al., 2013; Voigt et al., 2014). Conversely, a reduction in RyR expression is seen in several animal models of atrial tachycardia remodeling as part of the underlying proarrhythmic substrate (Lenaerts et al., 2009; Wakili et al., 2010; Lugenbiel et al., 2015).***"

3. RyR2 function is also increased in the atria of patients with HFrEF, despite the fact that total RyR2 levels were reduced (PMID: 30356673). This should mentioned.
Thank you, we added this (lines 498-500). ***"Notably, despite reduced total RyR protein levels in the atria of patients with systolic HF, RyR function was found to be increased (Molina et al., 2018)."***

4. LCC variation: Christ et al. 2004 do not show a decrease in LCC expression, although the current is reduced in AF patients.
Thank you, we corrected this (line 556).

Dear Ele,

Re: JP-RP-2022-283602R1 "Mechanisms of spontaneous Ca2+ release-mediated arrhythmia in a novel 3D human atrial myocyte model: II. Ca2+-handling protein variation" by Xianwei Zhang, Charlotte Smith, Stefano Morotti, Andrew Edwards, Daisuke Sato, William E. Louch, Haibo Ni, and Eleonora Grandi

I am pleased to tell you that your paper has been accepted for publication in The Journal of Physiology.

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Yours sincerely,

Bjorn Knollmann
Senior Editor
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authors DO NOT NEED to pay to publish and DO NOT NEED to post their accepted papers on PMC.

EDITOR COMMENTS

Reviewing Editor:

No further comments.

Senior Editor:

Wonderful study, congratulations!

1st Confidential Review **19-Aug-2022**