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ATP-sensitive potassium channels in zebrafish cardiac and vascular smooth muscle

Colin G Nichols, Soma S Singareddy, Helen I Roessler, Conor McClenaghan, Jennifer M Ikle, Robert Tryon, and Gijs van Haaften
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The following individual(s) involved in review of this submission have agreed to reveal their identity: William A. Coetzee (Referee #2)

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Dear Dr Nichols,

Re: JP-RP-2021-282157 "ATP-sensitive potassium channels in zebrafish cardiac and vascular smooth muscle" by Colin G Nichols, Soma S Singareddy, Helen I Roessler, Conor McClenaghan, Jennifer M Ikle, Robert Tryon, and Gijs van Haaften

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EDITOR COMMENTS

Reviewing Editor:

This is a carefully conducted and clearly described study characterizing the ATP-sensitive K+ channels found in cardiac

myocytes and vascular smooth muscle cells from the zebra fish. This work, while largely descriptive, establishes the zebra fish as a model system for future studies on the role of these channels in the cardiovascular system. The reviewers have just a few points that should be addressed.

Senior Editor:

Two Expert Referees and a Reviewing Editor voice interest in this study's strong potential to advance use of the zebrafish as a model organism for studying cardiovascular ATP-sensitive K channels. I found the manuscript to be thoughtful, well-written, and presented meticulously. Overall, the Referees and RE also concur on the manuscript's soundness and rigor, although Referee 1 suggests the Authors may wish to reconsider the statistical analyses performed. The Authors should have no problems addressing this, as well as a few other minor concerns. Some interesting questions are raised by both Referees; incorporation of thoughts on the different SUR isoforms and effects of ATP-sensitive K channel agonists might be entertained.

REFEREE COMMENTS

Referee #1:

This manuscript describes studies of KATP currents in inside-out patches of membrane from zebra fish ventricular and cardiac myocytes and whole-cell currents from vascular myocytes. Overall, the manuscript is well written and provides the first characterization of KATP channels in fish. Major concerns are listed below:

1. In several of your figures it appears that there is heterogeneity in the variance of the data (based on the SD's presented) suggesting that the use of parametric statistics (ANOVAs and t-tests), which assume homogeneity of variance, should not be performed. Please either transform the data to fix this problem or apply non-parametric statistics to you data. There also is some concern about the low n-values shown in the figures, particularly when borderline p-values are shown.

2. Your finding that the KATP channel agonists did not activate zebra fish KATP channels deserves some additional discussion. Do the protein sequences of the SURs and KATP channels in the fish provide any clues as to why these KCO's are without effect?

Minor concern - page 10, line 275 - Don't you really mean current density, rather than conductance in this sentence? Single channel conductance was not altered, correct? Please revise accordingly.

Referee #2:

Since 2006, when the Seino lab described a third member of the Kir6 subfamily in Zebrafish, little has been done to characterize KATP channels in this organism. The Nichols et al manuscript fills this gap in our knowledge with their description of Zebrafish cardiovascular KATP channels, which is long-overdue given the utility of this model organism to study human disease. The study is well executed and presented. I have only a few relatively minor comments:

The authors describe Kir6.2 and Zebrafish SUR2 expression and function in Zebrafish heart and vessels. A limitation of the study is that alternative isoforms of SUR2 are not considered. In human, SUR2A and SUR2B, which differ in the distal C-terminus as a result of alternative spicing events, are differentially expressed in ventricle and smooth muscle and also display differential pharmacological profiles. Inspection of Zebrafish RefSeq sequences shows that SUR2 has several different isoforms in Zebrafish, with at least one of these (e.g. XM_017355035.2) that differs in the distal C-terminus.

The present study does not consider the possible presence of SUR2 isoform expression in the Zebrafish cardiovascular system. This should, at the very minimum, be discussed.

The lack of effects of KATP channel openers on the Zebrafish KATP channel is intriguing. It is surprising that efforts have not been made to elucidate the possible molecular mechanisms of this finding. The authors should, at the minimum, discuss possible reasons, which may include sequence differences between human and Zebrafish SUR2. The recent AlphaFold structure predictions of Zebrafish proteins (e.g. https://www.alphafold.ebi.ac.uk/entry/Q5RH87) may go a long way to identify structural differences.

Page 10: 100 mM of pinacidil?

END OF COMMENTS

Confidential Review **05-Aug-2021**

Response to reviewer's comments (comments in black, responses in red)

Reviewing Editor:

This is a carefully conducted and clearly described study characterizing the ATP-sensitive K+ channels found in cardiac myocytes and vascular smooth muscle cells from the zebra fish. This work, while largely descriptive, establishes the zebra fish as a model system for future studies on the role of these channels in the cardiovascular system. The reviewers have just a few points that should be addressed.

We very much appreciate the positive reception from the Editors and reviewers. We have now addressed each point.

Senior Editor:

Two Expert Referees and a Reviewing Editor voice interest in this study's strong potential to advance use of the zebrafish as a model organism for studying cardiovascular ATP-sensitive K channels. I found the manuscript to be thoughtful, well-written, and presented meticulously. Overall, the Referees and RE also concur on the manuscript's soundness and rigor, although Referee 1 suggests the Authors may wish to reconsider the statistical analyses performed. The Authors should have no problems addressing this, as well as a few other minor concerns. Some interesting questions are raised by both Referees; incorporation of thoughts on the different SUR isoforms and effects of ATP-sensitive K channel agonists might be entertained.

Thank you. The statistical analyses have been redone. We have added further consideration of different SUR isoforms and KATP opener insensitivity.

Referee #1:

1. In several of your figures it appears that there is heterogeneity in the variance of the data (based on the SD's presented) suggesting that the use of parametric statistics (ANOVAs and t-tests), which assume homogeneity of variance, should not be performed. Please either transform the data to fix this problem or apply non-parametric statistics to you data. There also is some concern about the low n-values shown in the figures, particularly when borderline p-values are shown.

Statistical analyses have been re-performed using non-parametric tests as suggested, and where indicated.

2. Your finding that the KATP channel agonists did not activate zebrafish KATP channels deserves some additional discussion. Do the protein sequences of the SURs and KATP channels in the fish provide any clues as to why these KCO's are without effect?

This is an inte4resting point. Unfortunately, given that the KCO binding site in mammalian KATP channels is not yet structurally identified, it is difficult to draw parallels or get a concrete idea as to where the differences lie. As we now discuss, prior studies identified L1249 and T1253 as key residues conferring opener sensitivity in rat SUR2a. Mutation of residue M1290 in hamster SUR1 to Thr (eq. to residue 1253 in SUR2a) renders it fully activated by the other KCOs. Alignment of the zfSUR2A sequence, with the ratSUR2A

Colin G. Nichols FRS, Carl Cori Professor and Director, Center for Investigation of Membrane Excitability Diseases

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sequence shows that the equivalent residue is already Thr in ZF. Thus, it appears that additional unidentified residues may be involved in KCO binding. We have added further consideration of this point in the Discussion.

Minor concern - page 10, line 275 - Don't you really mean current density, rather than conductance in this sentence? Single channel conductance was not altered, correct? Please revise accordingly.
Yes, current density is a less ambiguous term. Revised as suggested.

Referee #2:
Since 2006, when the Seino lab described a third member of the Kir6 subfamily in Zebrafish, little has been done to characterize KATP channels in this organism. The Nichols et al manuscript fills this gap in our knowledge with their description of Zebrafish cardiovascular KATP channels, which is long-overdue given the utility of this model organism to study human disease. The study is well executed and presented. I have only a few relatively minor comments:
The authors describe Kir6.2 and Zebrafish SUR2 expression and function in Zebrafish heart and vessels. A limitation of the study is that alternative isoforms of SUR2 are not considered. In human, SUR2A and SUR2B, which differ in the distal C-terminus as a result of alternative spicing events, are differentially expressed in ventricle and smooth muscle and also display differential pharmacological profiles. Inspection of Zebrafish RefSeq sequences shows that SUR2 has several different isoforms in Zebrafish, with at least one of these (e.g. XM_017355035.2) that differs in the distal C-terminus.
The present study does not consider the possible presence of SUR2 isoform expression in the Zebrafish cardiovascular system. This should, at the very minimum, be discussed.
A preliminary transcript expression of the two isoforms was obtained using the RT-PCR study, but it is correct that alternative isoforms of SUR2 are not robustly characterized using our electrophysiological studies.. A common way to differentiate the two isoforms in mammals is via pharmacology (as SUR2B, but not SUR2A, is activatable by diazoxide). Given that we do not see KCO activation in either cardiac or vascular KATP, we cannot discriminate isoforms based on pharmacology. We have not tested DZX activation in ZF VSM cells in this study, because there is no control data to show whether zebrafish SUR2B is activatable by DZX. We have added further consideration of tissue-specificity of isoforms and the above imitations in the Discussion.

The lack of effects of KATP channel openers on the Zebrafish KATP channel is intriguing. It is surprising that efforts have not been made to elucidate the possible molecular mechanisms of this finding. The authors should, at the minimum, discuss possible reasons, which may include sequence differences between human and Zebrafish SUR2. The recent AlphaFold structure predictions of Zebrafish proteins
(e.g. https://www.alphafold.ebi.ac.uk/entry/Q5RH87) may go a long way to identify structural differences.
AlphaFold is an excellent suggestion and we have now examined this, allowing more extensive consideration of the possible reasons for zebrafish KATP insensitivity to KATP openers.

Page 10: 100 mM of pinacidil?

Thank you for pointing this typographical error. It is supposed to be 100 µM (0.1 mM). The revised manuscript has now been proofed to remove such errors.

Dear Dr Nichols,

Re: JP-RP-2021-282157R1 "ATP-sensitive potassium channels in zebrafish cardiac and vascular smooth muscle" by Colin G Nichols, Soma S Singareddy, Helen I Roessler, Conor McClenaghan, Jennifer M Ikle, Robert Tryon, and Gijs van Haaften

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Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

The authors have addressed all previous concerns.

Senior Editor:

The Authors thoroughly addressed all comments arising from initial review of their manuscript. Both Expert Referees and the Reviewing Editor agree that this study will be impactful. Please accept my congratulations on a job well done.

REFEREE COMMENTS

Referee #1:

The authors have adequately addressed my concerns. No additional comments.

Referee #2:

No further comments - the authors are to be congratulated on an excellent study.

1st Confidential Review **02-Nov-2021**