The Journal of Physiology

Mitochondrial carbonic anhydrase VA and VB: Properties and roles in health and disease

Ashok Aspatwar, Claudiu T. Supuran, Abdul Waheed, William S Sly, and Seppo Parkkila
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The referees have opted to remain anonymous.

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Reviewing Editor: Kyle McCommis

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision **25-Oct-2022**

Dear Professor Parkkila,

Re: JP-TR-2022-283579 "Mitochondrial carbonic anhydrase VA and VB: Properties and roles in health and disease" by Ashok Aspatwar, Claudiu T. Supuran, Abdul Waheed, William S Sly, and Seppo Parkkila

Thank you for submitting your Topical Review 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.

The reports are copied at the end of this email. Please address all of the points and incorporate all requested revisions, or explain in your Response to Referees why a change has not been made.

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I hope you will find the comments helpful and have no difficulty in revising your manuscript within 4 weeks.

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I look forward to receiving your revised submission.

Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

Thank you for submitting your review manuscript titled "Mitochondrial carbonic anhydrase VA and VB: Properties and roles in health and disease" to The Journal of Physiology. We must apologize for the lengthy delay, it required a substantial amount of time to secure 2 independent reviewers. This manuscript has now been assessed by 2 academic peer reviewers and an academic reviewing editor. I am pleased to say that all reviewers believe this review article to be very well written, and likely to have high influence on the field of metabolic physiology and rare diseases. Both reviewers identified minor issues in the writing and have several minor critiques which should be easy to address. We look forward to receiving your revised review article.

Please see 'required items' below.

REFEREE COMMENTS

Referee #1:

This is a well-written, concise and up-to-date review on the two mitochondrial carbonic anhydrase isoforms VA and VB, respectively. Physiology, pathophysiology, pharmacology and role in human disease are discussed.

I only have minor comments that may help to improve the manuscript:

1. Figures 4 and 8 could be improved in quality, design and color choice. Furthermore, the font size should be increased.

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3. GWAS: have any phenotypic traits been linked to SNPs in the region of the CA VA or CA VB genes ?

Referee #2:

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The presented review gives complete information on recently progress made in the field.

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1) Abstract figure: Rephrase last two lines regarding presence of CA VA and B, specify which are the organs where CA VB is present.

2) Pag 4, lane 14, an "and" is missing between VA VB.

3) Pag 5, Biochemistry CA VA and CA VB. It would be clearer to the reader if authors would insert a table regarding the catalytic efficiency of these two isoforms compared to the other isoforms, rather than listing them in the text.

4) Pag 6 and 7, Authors repeat twice the information regarding the amino acidic similarity of CA VB to CA VA (66%). Please edit.

5) Pag 7, Authors should include in the text the information that when protein numbering is not inclusive of the signal peptide, residue position is annotated with a + before the residue number. This can help the reader and avoid confusion when numbered residues are cited within the manuscript.

6) Pag 7, CA VB contains 6 cysteines and not 5 as mentioned in the text. Please correct the sentences in the text.

7) Pag 8, Fig 3, please box the cysteine in position 92 of CA VA and VB, and edit Figure legend.

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-Your MS must include a complete "Additional information section" with the following 4 headings and content:

Competing Interests: A statement regarding competing interests. If there are no competing interests, a statement to this effect must be included. All authors should disclose any conflict of interest in accordance with journal policy.

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END OF COMMENTS

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Professor Laura Bennet
Senior Editor
The Journal of Physiology
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UK

November 14th, 2022

Dear professor Bennet,

We were grateful for the constructive critiques our manuscript (JP-TR-2022-283579 "Mitochondrial carbonic anhydrase VA and VB: Properties and roles in health and disease") received and feel that we were able to respond to the questions or suggestions with changes that greatly improved our work. Below you can find the modifications made according to reviewers´ comments. We also corrected some typos and other errors found in the text during the editing process. I hope that you will find the revised version of the manuscript acceptable for publication in the Journal of Physiology.

Sincerely yours,

Seppo Parkkila, professor, dean
Faculty of Medicine and Health Technology
Tampere University, Finland

Reviewing Editor:

Thank you for submitting your review manuscript titled "Mitochondrial carbonic anhydrase VA and VB: Properties and roles in health and disease" to The Journal of Physiology. We must apologize for the lengthy delay, it required a substantial amount of time to secure 2 independent reviewers. This manuscript has now been assessed by 2 academic peer reviewers and an academic reviewing editor. I am pleased to say that all reviewers believe this review article to be very well written, and likely to have high influence on the field of metabolic physiology and rare diseases. Both reviewers identified minor issues in the writing and have several minor critiques which should be easy to address. We look forward to receiving your revised review article.

- We thank the Reviewing Editor for these very positive comments on our manuscript.

Reviewer #1

This is a well-written, concise and up-to-date review on the two mitochondrial carbonic anhydrase isoforms VA and VB, respectively. Physiology, pathophysiology, pharmacology and role in human disease are discussed.

I only have minor comments that may help to improve the manuscript:

1. Figures 4 and 8 could be improved in quality, design and color choice. Furthermore, the font size should be increased.
   Our response: The new versions of Figures 4 and 8 have been improved for better clarity.

2. The authors repeatedly mention the putative role of CA VB in male spermatogenesis and fertility, statements which are largely based on in vitro studies. But CA VB KO mice have been developed and characterized - some of the authors have been involved in these studies. No apparent phenotype of CA VB Ko mice was reported. Is there evidence that male CA VB KO mice exhibit reduced fertility ? If this is not the case, then this should be mentioned in the text.
   Our response: We have modified the last sentence of Conclusions as follows: " Recent inhibition studies involving Sertoli cells suggested that CA VB plays an important role in spermatogenesis, although no reproductive phenotype was observed in mice deficient only for CA VB."
3. GWAS: have any phenotypic traits been linked to SNPs in the region of the CA VA or CA VB genes ?
   Our response: We have added the following paragraph to the end of "Physiological functions of CA VA and CA VB" section (page 12): " GWAS Central database (https://www.gwascentral.org/index) can be searched to find genome-wide associations between single nucleotide polymorphisms (SNPs) and phenotypes. Using p-value threshold - log p≥2, there are two phenotypes significantly associated with markers in gene or region of the CA5A gene. The phenotype ontology annotation indicates a linkage to Alzheimer´s disease and breast cancer. At this p-value threshold, the CA5B gene shows no phenotypic associations."

Referee #2:

The paper by Aspatwar et al. entitled "Mitochondrial carbonic anhydrase VA and VB: Properties and roles in health and disease" is an interesting review regarding these two mentioned mitochondrial isoforms. The review is very well written and dissects all the aspects of these two isoforms, from the biochemical to the functional features and the inhibition studies carried out on the two enzymes.

The presented review gives complete information on recently progress made in the field.

Only some minor revisions are necessary:

1) Abstract figure: Rephrase last two lines regarding presence of CA VA and B, specify which are the organs where CA VB is present.
Our response: We thank the reviewer for this comment. We have modified the text for Abstract figure as follows: ” The liver hepatocytes are the main source of carbonic anhydrase VA with weaker signals in brain, testis, and muscle. The VB isozyme is more widely spread in several organs, such as brain, heart, liver, lung, kidney, spleen, intestine, testis, muscle, and pancreas.”

We also defined the corresponding section more precisely in Introduction: ”CA VA is mainly expressed in the liver, with some expression also observed by Western blot in the brain, testis, and skeletal muscle (Shah et al., 2000). The CA VB isozyme has a much wider tissue distribution and is expressed in the heart, liver, lung, spleen, intestine, pancreas, testis, skeletal muscle, kidney, salivary gland, brain, and spinal cord, suggesting different physiological roles for these two mitochondrial isozymes (Fujikawa-Adachi et al., 1999; Ghandour et al., 2000; Shah, et al., 2000).”

2) Pag 4, lane 14, an "and" is missing between VA VB.
Our response: Instead of adding ”and” we replaced VA and VB with ”CA activity” which is a more accurate term in this context (page 4).

3) Pag 5, Biochemistry CA VA and CA VB. It would be clearer to the reader if authors would insert a table regarding the catalytic efficiency of these two isoforms compared to the other isoforms, rather than listing them in the text.
Our response: We have added Table 1 according to the reviewer´s suggestion.

4) Pag 6 and 7, Authors repeat twice the information regarding the amino acid similarity of CA VB to CA VA (66%). Please edit.
Our response: We thank the reviewer for pointing out this repeated sentence. The first repeated sentence has been removed.

5) Pag 7, Authors should include in the text the information that when protein numbering is not inclusive of the signal peptide, residue position is annotated with a + before the residue number. This can help the reader and avoid confusion when numbered residues are cited within the manuscript.
Our response: The information requested by the reviewer has been added to the text in the ”Molecular biology of CA VA and CA VB” section (page 8).

6) Pag 7, CA VB contains 6 cysteines and not 5 as mentioned in the text. Please correct the sentences in the text.

Our response: We thank the reviewer for pointing out this error. The sentence about these cysteines has been corrected (Figure 3 legend).

7) Pag 8, Fig 3, please box the cysteine in position 92 of CA VA and VB, and edit Figure legend.
Our response: Referring to the previous comment, the sentence and figure has been corrected accordingly.

8) Pag 7 Authors state that CA VB may contain four Cys residues possibly involved in intradisulfide bond formation referring to reference 39 which is a PNAS manuscript on CA IV. Please note that in CA IV the cysteines involved into disulfide bonds are not conserved in CA VB. Please check the reference. Moreover, the crystallographic structure of murine CA V was solved showing that all the cysteine residues are reduced. Why Authors do not use this structure to predict the CA VB redox state of cysteines? Please check
Our response: We thank you the reviewer for pointing out the incorrect information about the cysteine residues of CA VB. We agree that the correlation to CA IV is not justified. Therefore, we deleted the incorrect statement and added the following (on page 8): "Although human CA VA and VB contain four and six cysteine residues, respectively, no disulfide bridges as post-translational modifications have been reported in these enzymes (Di Fiore et al. 2020)."

Dear Professor Parkkila,

Re: JP-TR-2022-283579R1 "Mitochondrial carbonic anhydrase VA and VB: Properties and roles in health and disease" by Ashok Aspatwar, Claudiu T. Supuran, Abdul Waheed, William S Sly, and Seppo Parkkila

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

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Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

Thank you for submitting your revised commissioned review article titled "Mitochondrial carbonic anhydrase VA and VB: Properties and roles in health and disease" to The Journal of Physiology. The two external peer reviewers and the academic

reviewing editor believe the changes made in response to reviewers' critiques have improved the manuscript greatly. Thank you.

REFEREE COMMENTS

Referee #1:

The authors addressed all my concerns adequately.

Referee #2:

The paper " Mitochondrial carbonic anhydrase VA and VB: Properties and roles in health and disease" can now be accepted for publication.

1st Confidential Review **20-Nov-2022**