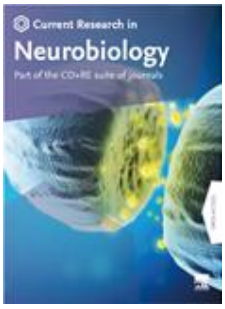
Peer Review Overview



Manuscript Title: Insulin-like growth factor-1 stimulates retinal cell proliferation via activation of multiple signaling pathways

Received	Jul 17, 2022
1st Decision	Aug 19, 2022
1st Revision Submitted	Sep 05, 2022
Accepted	Dec 12, 2022

1st Decision letter

Reference: CRNEUR-D-22-00096
Title: : Insulin-like growth factor-1 stimulates retinal cell proliferation via activation of multiple signaling pathways
Journal: Current Research in Neurobiology

Dear Mrs Giestal-de-Araujo,

Thank you for submitting your manuscript to Current Research in Neurobiology.

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following minor revision and modification. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Oct 18, 2022.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

Current Research in Neurobiology values your contribution and I look forward to receiving your revised manuscript.

CRNEUR aims to be a unique, community-led journal, as highlighted in the Editorial Introduction. As part of this vision, we will be regularly seeking input from the scientific community and encourage you and your co-authors to take the survey.

Kind regards,

Abhishek Banerjee
Associate Editor
Current Research in Neurobiology

Comments from Editors and Reviewers:

Reviewer #1:

The authors submitted the "Short Communication (for Double-Anonymized Review)" with the title "Insulin-like growth factor-1 stimulates retinal cell proliferation via activation of multiple signaling pathways" to the journal "Current Research in Neurobiology".

The article describes, in brief, different pathways of Insulin-like growth factor-1 (IGF-1) for retinal proliferation in rat primary cultures. Briefly, the authors use primary culture rat retinal cells and a pharmacological approach to dissect the different pathways of IGF-1-induced retinal cell proliferation. In summary, they were able to show that IGF-1-induced retinal cell proliferation is depending on several pathways, including the IGF-1R/PI3K/AKT, PKCδ, PLC, JNK, and MAPK/ERK pathways.

IGF-1 is an important growth hormone and plays an important role in the development of the central nervous system. It has also been shown that IGF-1 is also instrumental in the formation of the retina and 3D retinal organoids. Similar pathways have been revealed for fish, mice and human but not rats. Hence, the knowledge of IGF-1 pathways for retinal cell proliferation in rats is suitable for a short communication in Current Research in Neurobiology. The article is well written, the figures are clearly structured, and the conclusions drawn from the results/statistics are sound and correct. Therefore, I endorse publication after minor revisions.

The minor revisions are as follows:
1. As stated above, similar pathways are known in other animal models and human. It would make the article stronger if these articles are already mentioned in the introduction conjoined with a clearer explanation of the novelty in this study.

2. The Methods are well written and the study is reproducible. However, the Ethical Statement is missing from the version I have reviewed. Please add information about the animal groups (see guide journal guidelines).

3. The results are well presented but could do with some clarifications as the journal's readers come across the broad spectrum of neuroscience. For example, on page 4 mention in lay terms what a [3H]-thymidine assay measures - extent of cell division/ proliferation.

4. Figures: Fig 1B could benefit from a log x axis. In Fig 1C include 10ng/48 hrs rather than refer to Fig 1A - It will help to compare. In Fig2B and ff, use either the name of the inhibitor or what is inhibited - It should be consistent.

Reviewer #2:

The manuscript has a novel concept of role of IGf-1 in retinal cell development. The article is well written but more elaborate explanation about the role of IGF-1 in rat species may be included in introduction. The whole data relies on the cultured retinal cells but there is not a single photograph of cells. Figure 1 which describes viability of cells on the basis of tritium incorporation should be supported by photomicrographs pf cells. The role of IGF-1 is established by checking the involvement of all signalling molecules which mediates the effect of IGF-1 on the development of retinal cells.The

comprehensive approach of using the inhibitors to MEK-ERK, p38-MAPK and JNK to unraveil the signalling pathway is good but the results of immunoblotting could have been supported by immunohistochemistry or mRNA expressions. The signalling molecules MAP kinase and PI-3 kinase that mediates the role of IGF-1 in retinal cell proliferation is already reported but reestablishment of the fact taking mutants of Igf-1 and Igf-1R was a good idea. Moreover a toal picture of the signalling mechanism evolved which was appreciating. IGF-1 considered as proangiogenic molecule in retinal degenerations like diabetic retinopathy has regained it's significance through the studies highlighting it's beneficial effects. The involvement of EGFR and IL-4 in IGF-1 mediated retinal cell proliferation is also well documented. Since there are many reports of proaniogenic behavior of IGF-1 in degenerations like diabetic retinopathy, the discussion portion should include the interlinks of IGF-1 with expression pattern of VEGF, VEGFR1 and VEGFR2.
The parameters included in the study are tritium incorporation for viability and western blotting for explaining the signalling mechanisms involved.The protein level expressions could be reinforced by seeing mRNA level expressions through RT- PCR.

1st Author Response Letter

Response to comments from Editors and Reviewers:

Comments from Reviewer 1

The authors submitted the "Short Communication (for Double-Anonymized Review)" with the title "Insulin-like growth factor-1 stimulates retinal cell proliferation via activation of multiple signaling pathways" to the journal "Current Research in Neurobiology". The article describes, in brief, different pathways of Insulin-like growth factor-1 (IGF-1) for retinal proliferation in rat primary cultures. Briefly, the authors use primary culture rat retinal cells and a pharmacological approach to dissect the different pathways of IGF-1-induced retinal cell proliferation. In summary, they were able to show that IGF-1-induced retinal cell proliferation is depending on several pathways, including the IGF-1R/PI3K/AKT, PKCδ, PLC, JNK, and MAPK/ERK pathways. IGF-1 is an important growth hormone and plays an important role in the development of the central nervous system. It has also been shown that IGF-1 is also instrumental in the formation of the retina and 3D retinal organoids. Similar pathways have been revealed for fish, mice and human but not rats. Hence, the knowledge of IGF-1 pathways for retinal cell proliferation in rats is suitable for a short communication in Current Research in Neurobiology. The article is well written, the figures are clearly structured, and the conclusions drawn from the results/statistics are sound and correct. Therefore, I endorse publication after minor revisions.

The minor revisions are as follows:

1. As stated above, similar pathways are known in other animal models and human. It would make the article stronger if these articles are already mentioned in the introduction conjoined with a clearer explanation of the novelty in this study.

R: We thank the reviewer for this comment. To the best of our knowledge, there are no previous studies demonstrating the signaling mechanisms by which IGF-1 stimulates retinal cell proliferation. Although Wang et al. (2018) reported that combined treatment with IGF-1 and EGF stimulates retinal cell proliferation in mice by activation of PI3K/AKT and MAPK/ERK pathways, no effect of IGF without EGF in

retinal cell proliferation was reported. Other studies have shown that IGF-1 acts as a mitogen in the fish retina (Becker et al., 2021; Otteson et al., 2002; Zygar et al., 2005) and human retinal organoids (Mellough et al., 2015; Zerti et al., 2021). However, none of these studies investigated molecular mechanisms by which IGF-1 stimulates retinal cell proliferation. Therefore, the main novelty of the present work is to identify signaling pathways by which IGF-1 ensures retinal cell proliferation. As a model, we used primary cultures from rat retinas. We have mentioned these studies in the Introduction with a brief explanation of the novelty (Page 2, lines 28-33 and 39-41).

References

Becker, C., et al. (2021). *Development*, 148(7), dev199133. doi.org/10.1242/dev.199133

Mellough, C. B., et al. (2015). *Stem Cells*, 33(8), 2416-2430. doi.org/10.1002/stem.2023

Otteson, D. C., et al. (2002). *Mech Dev*, 117(1-2), 137-149. doi.org/10.1016/S0925-4773(02)00188-0

Wang, Y., et al. (2018). *Cell Cycle*, 17(4), 515-526. doi.org/10.1080/15384101.2018.1431594

Zerti, D., et al. (2021). *Stem Cells*, 39(4), 458-466. doi.org/10.1002/stem.3331

Zygar, C. A., et al. (2005). *Dev Brain Res*, 154(1), 91-100. doi.org/10.1016/j.devbrainres.2004.10.009

2. The Methods are well written and the study is reproducible. However, the Ethical Statement is missing from the version I have reviewed. Please add information about the animal groups (see guide journal guidelines).

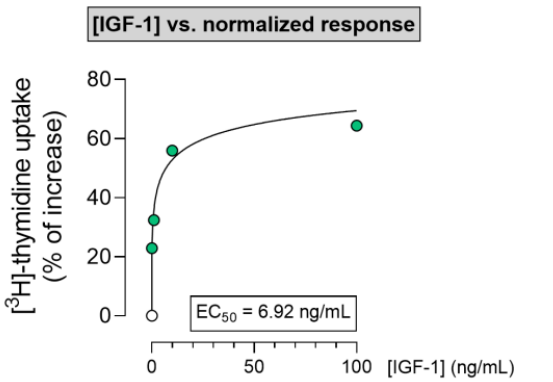
R: We thank the reviewer for raising this point. We carefully revised the Methods section to include the details indicated by the reviewer (Page 3, lines 62-70).

3. The results are well presented but could do with some clarifications as the journal's readers come across the broad spectrum of neuroscience. For example, on page 4 mention in lay terms what a [3H]-thymidine assay measures - extent of cell division/ proliferation.

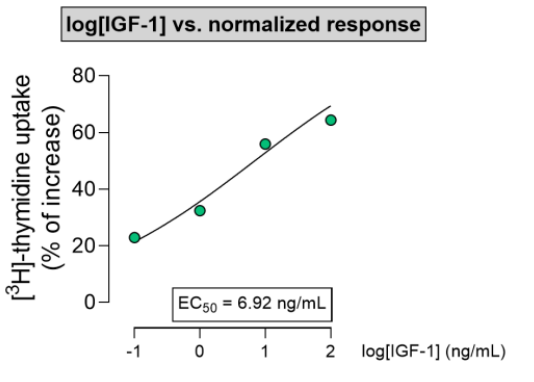
R: We thank the reviewer for this suggestion. We have added a sentence in Page 5 (lines 114-116) to mention this.

4. Figures: Fig 1B could benefit from a log x axis. In Fig 1C include 10ng/48 hrs rather than refer to Fig 1A - It will help to compare. In Fig2B and ff, use either the name of the inhibitor or what is inhibited - It should be consistent.

R: We thank the reviewer for positive and constructive comments on our manuscript. First, we performed a new dose-response graphic using log[IGF-1] as the x-axis. Nonetheless, we believe that the dose-response curve with [IGF-1] as the x-axis better represents the results reported in Fig 1A, please see Reviewer Fig 1 below. Second, we agree with the reviewer that including “10 ng/48 hrs” in Fig 1C would help to compare the results. However, Fig 1A and Fig 1C represent different experiments; therefore, we find it more appropriate not to duplicate “the 10ng/48 hrs result” in Fig 1C. Third, we have revised the Results section to provide more consistency in describing data and help to compare the results (Page 5, lines 120-122; Page 6, lines 148-149).



[Agonist] vs. normalized response -- Variable slope	
Best-fit values	
HillSlope	0.3070
EC50	6.916
logEC50	0.8399
95% CI (profile likelihood)	
HillSlope	0.1880 to 0.4490
EC50	3.251 to 18.06
logEC50	0.5120 to 1.257
Goodness of Fit	
Degrees of Freedom	46
R squared	0.6317
Sum of Squares	12637
Sy.x	16.57
Constraints	
EC50	EC50 > 0
Number of points	
# of X values	80
# Y values analyzed	48



log(agonist) vs. normalized response -- Variable slope	
Best-fit values	
LogEC50	0.8399
HillSlope	0.3070
EC50	6.916
95% CI (profile likelihood)	
LogEC50	0.4768 to 1.317
HillSlope	0.1762 to 0.4661
EC50	2.998 to 20.73
Goodness of Fit	
Degrees of Freedom	38
R squared	0.4073
Sum of Squares	12637
Sy.x	18.24
Number of points	
# of X values	64
# Y values analyzed	40

Reviewer Fig 1. Comparison between dose-response curves using [IGF-1] (left) or log[IGF-1] (right) as x axis.

Comments from Reviewer 2

The manuscript has a novel concept of role of IGf-1 in retinal cell development. The article is well written but more elaborate explanation about the role of IGF-1 in rat species may be included in introduction.

R: We thank the reviewer for this suggestion. We modified the Introduction section accordingly (Page 2, lines 34-37).

The whole data relies on the cultured retinal cells but there is not a single photograph of cells. Figure 1 which describes viability of cells on the basis of tritium incorporation should be supported by photomicrographs of cells.

R: We thank the reviewer for this comment. We have added representative photomicrographs of cells in new Fig 1E.

The role of IGF-1 is established by checking the involvement of all signalling molecules which mediates the effect of IGF-1 on the development of retinal cells. The comprehensive approach of using the

inhibitors to MEK-ERK, p38-MAPK and JNK to unraveil the signalling pathway is good but the results of immunoblotting could have been supported by immunohistochemistry or mRNA expressions.

R: We appreciate the reviewer's suggestion and agree that confirming the results of immunoblotting for IL-4 and BDNF with an additional approach would strengthen our conclusions. However, we feel that this additional investigation is beyond the scope of the current brief report. These experiments would take several additional months (reagent orders, rat breeding, culture preparation, and experimentation itself) and would surpass by far the revision deadline for this manuscript. Nonetheless, we continue to investigate these questions and hope to provide robust new data in the future. In addition, we have previously reported similar results for IL-4 levels upon IGF-1 treatment in primary retinal cells using ELISA and immunoblotting analysis (Granja et al, 2019). We mentioned these previous findings in Results (Page 5, lines 127-129) and Discussion (Page 7, lines 178-180).

The signalling molecules MAP kinase and PI-3 kinase that mediates the role of IGF-1 in retinal cell proliferation is already reported but reestablishment of the fact taking mutants of Igf-1 and Igf-1R was a good idea. Moreover a toal picture of the signalling mechanism evolved which was appreciating. IGF-1 considered as proangiogenic molecule in retinal degenerations like diabetic retinopathy has regained it's significance through the studies highlighting it's beneficial effects. The involvement of EGFR and IL-4 in IGF-1 mediated retinal cell proliferation is also well documented. Since there are many reports of proaniogenic behavior of IGF-1 in degenerations like diabetic retinopathy, the discussion portion should include the interlinks of IGF-1 with expression pattern of VEGF, VEGFR1 and VEGFR2.

R: We are grateful to the reviewer for their comments and suggestions. To the best of our knowledge, there are no previous reports showing the signaling mechanisms by which IGF-1 stimulates cell proliferation in the neural retina, including the involvement of EGF and IL-4. This is the novelty of the current study. We added a few sentences in the manuscript to discuss the potential role of VEGF in IGF-1-induced retinal cell proliferation (Page 7, lines 182-186)

Accept Letter

Dear Mrs Giestal-de-Araujo,

Thank you for submitting your manuscript to Current Research in Neurobiology.

I am pleased to inform you that your manuscript has been accepted for publication.

My comments, and any reviewer comments, are below.

Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate and value your contribution to Current Research in Neurobiology. We regularly invite authors of recently published manuscript to participate in the peer review process. If you were not already part of the journal's reviewer pool, you have now been added to it. We look forward to your continued participation in our journal, and we hope you will consider us again for future submissions.

CRNEUR aims to be a unique, community-led journal, as highlighted in the Editorial Introduction. As part of this vision, we will be regularly seeking input from the scientific community and encourage you and your co-authors to take the survey.

We would also like to invite you to take part in our CRNEUR Author Question & Answer (Q&A), which could get published alongside your article and help to promote it. We suspect you might have an interesting story of perseverance or team work that was required for the research study to complete, or a diversity of perspectives that you might share, as a way of inspiring others about neuroscience.

Kind regards,

Abhishek Banerjee
Associate Editor
Current Research in Neurobiology

Editor and Reviewer comments:

Reviewer 1: The authors have answered all reviewer questions. I endorse publication

-------- *End of Review Comments* --------