Author's response to reviews

Title: Activation of k-opioid receptor by U50,488H improves vascular dysfunction in streptozotocin-induced diabetic rats

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Author's response to reviews: see over
Dec 24, 2014

Dear editor,

Enclosed please find our revised manuscript (Manuscript ID: 5010287081455014) entitled “Activation of κ-opioid receptor by U50,488H improves vascular dysfunction in streptozotocin-induced diabetic rats” to *BMC Endocrine Disorders*.

We would like to appreciate your office and all the reviewers for their careful and thorough reading of our manuscript. We appreciate all the constructive comments and suggestions from the two reviewers, which are extremely helpful for us to improve the quality of this work. Together with the other authors, we have considered all the comments carefully and revised the manuscript accordingly. In this revision, we have copyedited the manuscript to minimize typographical, grammatical, and bibliographical errors by a native English speaker. We have also responded point by point to each reviewer’s comments as listed below, and clearly indicated the location of the revision in the manuscript.

We hope that the revised manuscript is acceptable for publication and look forward to your positive response.

We assure that this manuscript is not under consideration elsewhere and none of its contents was published previously. All authors have read and approved the final version of the revised manuscript.
Conflict of interest: none declared.

Thank you for your attention and consideration.

Sincerely yours,

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Response to Reviewers’ Comments

We are grateful to the reviewers for their helpful comments and suggestions regarding our manuscript. We have performed additional experiments and made all feasible revisions as suggested by the reviewers. A copy of the comments (highlighted in grey) followed by a detailed response can be found here. All changes and additions in the manuscript are highlighted in red for ease of review.

Reply to Reviewer: Gustavo Jorge Santos

1. The paper has important informations and have to have the English grammar revised, since there are grotesque mistakes in the text.

Response: We greatly appreciated these constructive suggestions from the reviewer. The revised manuscript has been copyedited by a native English speaker.

2. The Figures legend should be revised, since they are superficial.

Response: We wish to thank the reviewer for your constructive suggestion. The figures legend has been rewritten in the revised manuscript (page 23-25).

Reply to Reviewer: Fernanda Ortis

1. The English need to be revised.

Response: We thank the reviewer for the constructive suggestion. The revised manuscript has been copyedited by a native English speaker.

2. Since the model used doesn’t involves obesity (type 2 Diabetes) or autoimmunity (type 1 Diabetes) it should be interesting to mention this in the discussion, which indicate that the beneficial effects of the drug are related to the deleterious effect of
the hyperglycemia.

**Response:** We greatly appreciate the reviewer for the constructive suggestion. There are two main types of diabetes: insulin-dependent DM (type 1) and non-insulin-dependent DM (type 2). Type 1 DM is strongly associated with autoimmunity, while type 2 DM is strongly associated with obesity and insulin resistance. STZ-induced DM offers a very cost effective and expeditious technique for DM research. STZ also offers the additional benefit of being able to select specific traits of interest, which can be important for specific experimental design. In this study, the DM model was induced by intraperitoneal injection of STZ (35 mg/kg) for 3 days, which was useful to examine the beneficial effects of U50,488H against high glucose-induced vascular dysfunction *in vivo*, and could be devoid of potential confounding effects of obesity (type 2 DM) or autoimmunity (type 1 DM). In the revised version, we have expanded the discussion on page 14, line 17-22 and page 15, line 1-4 accordingly.

3. In figure 1, what is the negative control? Is there any tissue in this control? For the KOR staining, where is this protein being expressed? Could it be pointed in the picture? Was the expression of KOR tested in the presence of the U50,488H group?

**Response:** We thank the reviewer for your constructive points and apologize for the mistake. We have performed additional experiments accordingly to test the KOR expression in thoracic aortas in rats. Negative control was obtained by omission of the primary antibody, on the use of preimmune serum. As shown in Fig. 1A, KOR was mainly detectable in the endothelium and smooth muscle of the thoracic aortas, which has been highlighted with arrows in the revised version. We further tested the KOR expression in the DM+U50,488H group and found that there was no statistically significant difference compared with the DM group. We have revised the results (Figure 1) and the description of results on page 11, line 4-6.
4. In figure 2, 4 and 5, DM + U50 and DM + nor-BNI are statically different from control or only from DM?

**Response:** We thank the reviewer for this point. In this study, rats were randomly divided into CON, DM, DM+vehicle, DM+U50,488H and DM+nor-BNI groups. After the DM rats received daily treatment of U50,488H for 4 weeks, it showed that the vascular dysfunction (Figure 2), endothelial dysfunction (Figure 4), and chronic inflammation (Figure 5) were improved, but not restored to the normal levels. In addition, after the DM rats received daily treatment of nor-BNI for 4 weeks, the vascular dysfunction (figure 2), endothelial dysfunction (figure 4), chronic inflammation (figure 5) were further aggravated, which were significantly different from the CON group. We have revised the results (Figure 2, 4, 5) and the description of results accordingly (page 11, line 17-19; page 12, line 3-5; page 13, line 12-14, 21-22.)

5. Again in figure 2, the curve in B is for NE treatment, however in results is described to be for PE, this should be corrected.

**Response:** We wish to thank the reviewer for catching this mistake. We have replaced “PE” with “NE” in the revised manuscript (page 11, line 11).

6. In figure 5, is this western performed for nuclear proteins, as described in results, and if so, why is actin used as a loading control? If the western is performed for total protein content, as described in MM and also in discussion as increase in protein expression (not migration to the nucleus as in results), then the text also need to be corrected.

**Response:** We thank the reviewer for catching this and wish to express our apology for the inaccurate statement in the original version of manuscript. In this study, NF-κB p65 was detected in nuclear proteins. Nuclear extracts (for NF-κB p65) were
isolated using a nuclear NE-PER extract kit (Thermo Scientific, IL, USA). Equal amounts of nuclear extracts were loaded using histone 3 as internal controls. We have revised the methods (page 9, line 10-12, 22), results (Figure 5) and discussion accordingly (page 18, line 2-4).