

October 08, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (12562 - Review)

Title: Role of opioid receptor heterodimerization in pain modulation and tolerance development

Author: Annu Mudgal, Santosh Pasha

Name of Journal: World Journal of Pharmacology

ESPS Manuscript NO: 12562

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated
2. Revision has been made according to the suggestions of the reviewers

REVIEWER - 504942

This review paper has shown recent progress in the studies on opioid receptor heteromers and the effects of ligands on the receptors. The authors have suggested the advantageous effects of dualsteric ligands. This paper sheds light on pharmacology of opioid receptor heteromers and clinically relevant aspects. However, there are two major problems and a number of minor problems in the manuscript as follows:

Major Points:

Comment 1. Integrated schema regarding trafficking of opioid receptor homomers/heteromers and opioid receptor/other GPCR heteromers and interaction between their receptors and ligands and intracellular molecules should be shown.

Response: As suggested by the reviewer we have now included the integrated scheme in diagrammatic form regarding trafficking of opioid receptor heteromers in figure 3.

Comment 2. Pharmacology of splice variants of mu-opioid receptors should be mentioned. Furthermore, the mention regarding heteromerization of the variants and other opioid receptors should be added.

Response: The reviewer is right, but this has been purposely avoided for the sake of simplicity to keep the text simple and clear. Since MOR, KOR and DOR are the three major receptor variants of opioid receptors and they are majorly available, that is why they are mainly focussed.

Minor Points:

Comment: P. 1, line 4: The running title should be revised.

Response: The title has been revised

Comment: Each footer: Please number the pages of the manuscript.

Response: *The change has been made*

Abstract

Comment: P. 2, Line 3: The words, receptor and GPCR should be changed into receptors and GPCRs, respectively.

Response: *The change has been made*

Comment: Line 4: Use of hyphen should be unified. There are so many errors in the manuscript.

Response: *The change has been made*

Comment: Line 8: One space should be deleted.

Response: *The change has been made*

Comment: Line 9: the word of receptor should be added (i.e. receptor heteromers).

Response: *The change has been made*

Comment: Line 12: GPCRs should be changed into receptors. (p. 9, the second paragraph, line 12)

Response: *The change has been made*

Keywords:

Comment: Opiate tolerance should be changed into opioid tolerance.

Response: *The change has been made*

Comment: Tolerance free antinociception should be revised.

Response: *The change has been made*

Abbreviations

Comment: DOR(DOPr), KOR(KOPr) and MOR(MOPr) should be consistently used.

Response: *The change has been made*

Comment: NPFF, BRET, and MERF should be added and properly used in the text.

Response: *The change has been made*

Comment: DRG should be deleted.

Response: *The change has been made*

Comment: Furthermore, the abbreviations in the text should be carefully used. There are so many errors.

Response: *The change has been made*

Comment: P. 3, Introduction section

Line 2: reward processing should be added.

Response: *The word has been added*

Comment: Line 6: seven transmembrane spanning proteins should be used.

Response: *The word has been added*

Comment: Line 8: several mechanisms among these should be mentioned.

Response: *The mechanisms have been mentioned*

Comment: p. 4, the second paragraph, line 7: reference numbers should be added.

Response: *The reference numbers have been added*

Comment: p. 5, line 2: site should be used instead of area.

Response: *The change has been made*

Comment: p. 5, line 12: D of KD is subscript. KD should be specified.

Response: *The subscript has been specified in abbreviations section*

Comment: p. 5, line 14: Dc should be specified.

Response: *The term has been specified in abbreviations section*

Comment: p. 5, line 17: publications should be revised.

Response: *The change has been made*

Comment: p. 6, line 1: The easier detection should be specified.

Response: *The easier detection has been specified*

Comment: P. 6, line 8: enhancement of ligand binding and signal activity by antagonists should be specified.

Response: *The change has been made*

Comment: P. 6, line 9: the sentence should be revised. Other pathologies should be explained..

Response: *The sentence has been revised and other pathologies mentioned*

Comment: p. 6, the second paragraph, line 1: dependence should be added.

Response: *The change has been made*

Comment: p. 6, the second paragraph, line 4: sub-cellular level should be revised.

Response: *The change has been made*

Comment: p. 7, line 6: CCK8-S,-----SO H.--NH should be corrected.

Response: *The change has been made*

Comment: p. 7, figure 1: rectangle and ellipse should be explained. Anti-opioid peptides and anti-opioid receptor should be specified.

Response: *The change has been made*

Comment: p. 9, the second paragraph, line 10: (DRG) should be deleted.

Response: *The change has been made*

Comment: p. 10, lines 5 and 6: (FCS) and (N&B) should be deleted.

Response: *The change has been made*

Comment: p. 11, line 1: some behavioural effects should be specified.

Response: *The change has been made*

Comment: p. 11, the second paragraph, line 6: CNS should be specified.

Response: *The change has been made*

Comment: p. 11, the second paragraph, line 8: a ligand should be specified, and the sentence should be revised.

Response: *The change has been made*

Comment: p. 12, the second paragraph: the sentences should be revised.

Response: *The change has been made*

Comment: p. 13, line 10: redMOR/greenDOR should be specified.

Response: *The change has been made*

Comment: p. 13, line 16: sub cortical should be corrected.

Response: *The change has been made*

Comment: p. 13, line 19: the sentence should be revised.

Response: *The change has been made*

Comment: p. 13, the second paragraph, line 2: anti-opioid receptors should be specified.

Response: *The change has been made*

Comment: p. 14, line 3: Roman alphabet, d, should be changed into Greek alphabet.

Response: *The change has been made*

Comment: p. 15, the second paragraph: the caption should be revised.

Response: *The change has been made*

Comment: p. 15, the second paragraph, line 3: opioid GPCRs should be revised.

Response: *The change has been made*

Comment: p. 21, line 1: i/o of Gai/o should be corrected. Since there are many errors like this, the authors should carefully revise the manuscript.

Response: *The change has been made*

Comment: p. 22, line 8: assays (---test) should be revised.

Response: *The change has been made*

Comment: p. 25: (IP), (GPI), and (MVD) should be deleted.

Response: *The change has been made*

Comment: p. 26, the second paragraph, line 11: in vivo should be revised.

Response: *The word in vivo has been revised*

Comment: p. 27, the second paragraph, line 13: z of Gaz should be corrected.

Response: *The term has been corrected*

Comment: p. 28, the fourth paragraph, line 3: Medicine should be corrected.

Response: *The change has been made*

Comment: p. 30, lines 4-: Ca²⁺ should be corrected.

Response: *The change has been made*

Comment: p. 31-33: beta should be corrected.

Response: *The change has been made*

References

Comment: The references should be corrected according to the journal formatting.

Response: *The change has been made*

Comment: TABLE 1

Reference number in the Ref column should be added.

Response: *The change has been made*

Comment: Many words are split.

Response: *The change has been made*

REVIEWER – 699925

The present review highlights the heteromerization process of the specific opioid receptors mu-delta already described in the literature, as well as the importance of developing pharmacological analogs (agonists and antagonists) with simultaneous “dual” activity on such receptors. As proposed, compounds presenting such dual activity would allow a more specific control of pain conditions without the related adverse effects of desensitization and tolerance that compromise the use of the most important analgesics currently available for the treatment of chronic pain, i.e., the opioid analgesics. Considering “Contents” as the first review page, deficiencies, eventual substitutions and suggestions are indicated below:

Deficiencies and substitutions:

Comment 1: Heteromerization is not only associated with opioid receptors.

Response: *The reviewer is right, but our focus is only opioid receptors.*

Comment 2: Lack of a glossary to explain or define all acronyms and important terms presented. Exemplifying: KD and Dc (page 3), FRET and BRET (page 8), NPFF (page 11); MERF (page 22); NTB (page 27).

Response: *All the important terms have been added in abbreviations section*

Comment 3: Terms such as oligomerization, protomer, tolerance, anti-opioid system should be provided and follow IASP definitions, fixing the terms afterwards. Opioid receptors have been interchangeable all through the text as: MOR, DOR, KOR; MOPr, DOPr, KOPr, as were MOP-DOP, MOR-DOR, MOR/DOR, and m-d heteromers (page 10) that difficult the flow of the reading.

Response: *The Change has been made*

Comment 4: A phrase is missing before “Intracellularly, the two receptors may also interact physically, and operate as homo- or heteromers with...”(page 7)

Response: *The sentence has been duly revised*

Comment 5: Indication of important references is lacking along text or they simply don't exist. Ex: for NPFF in the second paragraph (page 6) also, in vivo co-localization of MOR/DOR in rostro-ventral medulla (page 7). Should it be reference 29 lacking at the end of the first paragraph? (page 8). Yet, in the same page, a phrase in the second paragraph (“However, other studies found no such interactions”) should finish with a reference or references. Moreover, reference #100 is indicated after #101 on page 32. On the other hand, identification of the references Yekkirala et al., 2013 and Gupta et al., 1999 on pages 21 and 32, respectively, are unnecessary.

Response: *The Change has been made*

Comments and suggestions:

Comment 1: Evolutionary development of an anti-opioid system in superior animals certainly has been acquired to counterbalance an excessive (endogenous) opioid tonus. Can't it be expected that blocking partially or an integral part of such a system with dual compounds as proposed would compromise the proper functioning of the whole system? In other words, it would mean development of yet unknown adverse side effects associated with proposed compound's, a subject not discussed in the review.

Response: *The reviewer's point of concern is very obvious, but such dualsteric ligands should not actually raise any concerns. The purpose behind their conception and designing is to combat the tolerance development due to chronic administration of a drug. Such ligands will bind to opioids side to exhibit their opioid effect and their binding to anti-opioid side will lower the chances of tolerance development. (Elhabazi K, Simonin F, et.al. Involvement of neuropeptide FF receptors in neuroadaptive responses to acute and chronic opiate treatments. Br J Pharmacol 2012; 165(2): 424-35).*

Comment 2: In some places the text doesn't flow rationally. For instance, there is an interval in the text between the description of the anti-opioid system and the proposed dual compounds, leaving the intent (of the review) in the air. Moreover, illustration in Figure 1, which is supposed to help the understanding of the whole system's functioning doesn't give information on “who is who” in the balance (white rectangle= opioid system?). Besides, the upper arrow shouldn't be bidirectional?

Response: *The description has been revised and the change has been made in the figure*

Comment 3: Considering a strict chemical terminology, the term “bivalent” ligands in the illustration of Fig. 2 wouldn't be better to be substituted by “dual” ligands? The term “bivalent” is also used in the phrase “In short, as all bivalent ligands described to date...” (page 24) instead of dual ligands.

Response: *The term bivalent has been replaced by dual*

Comment 4: The term “Resensitization” under an arrow at the left front in illustration of Figure 3 could be substituted by reallocation (in the membrane). Resensitization is the consequence of receptor re-integralization in the membrane.

Response: *The change has been made in the figure*

Comment 5: The sequence of phrases in conclusion is truncated. Suggestions: “Research on opioid receptors has been...” - phrase 1. “The discovery of opioid receptor heteromers has...” - phrase 2. “This review highlighted the dualsteric...” - phrase 3. “A better understanding of these...” - phrase 4.

Response: *The conclusion section has been revised*

Comment 6: A complete review of the references is necessary as author's names are wrong as in Ref. 6 (Rozenfield instead of Rozenfeld); article's title is defective as in ref. 16 (...heterodimerization of and...). Also, full data are missing in refs 24, 44, 47, 74, 97.

Response: *The changes in the references has been made*

Comment 7: Table at the text end is not quoted on the text body.

Response: *We regret this and have now mentioned the table in the text under caption "Pharmacology and Therapeutic potential of opioid receptor heteromers"*

Comment 8: Minor points: a) exchange word "confirmation" to "conformation" (page 3); b) substitute the word "chronic" by "continuous" (first paragraph on page 3) and improve the phrase on definition of "Neuropeptide FF..." (2nd paragraph, same page). c) Improve the 3rd paragraph on page 10 ("One of the studies provides novel insights into the trafficking ...") d) Part of the text on page 11 was extracted "ipsis litteris" from the cited reference abstract (reference #10). e) A selective antibody against MOPr-DOPr is not identified on page 20, although it constituted a strong argument on the specific text (page 20). f) It lacks an "a" at the end of the phrase "These results indicated that this amphiphilic peptide has (a) role in pain modulation." G) "The phrase "the opioid receptor subfamily comprises mu, delta..." is unnecessary on the first paragraph of page 24.

Response: *The changes have been made*

4. References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Pharmacology*.

Sincerely yours,

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