Reviewer's report

**Title:** Label-free integrative pharmacology on-target of opioid ligands at the opioid receptor family

**Version:** 2  **Date:** 28 July 2012

**Reviewer:** Richard M van Rijn

**Reviewer's report:**

Morse et al apply a recently developed label-free integrative pharmacology on target approach (iPOT) to investigate the functional selectivity of a library of opioid ligands at the opioid receptors. This approach offers a broad platform for drug development focused on functional selectivity. The authors use a human cell line (HEK) overexpressing mu (MOR), delta (DOR) or kappa (KOR) opioid receptors and a human neuroblastoma cell line (SH-SY5Y) endogenously expressing the opioid receptors and test the dynamic mass redistribution induced by a library of opioid selective compounds. Some important findings are: 1) the identification of off-target opioid effects 2) identification of true neutral antagonists 3) evidence that opioid ligands behave differently in HEK cells artificially expressing only one opioid receptor vs cell endogenously expressing opioid receptors 4) data that suggests that kappa opioid receptors is a stronger mediator of p38 MAPK than other opioid receptors.

I believe the findings and the quality of the work are suitable for publication in BMC Clinical Pharmacology. However, there are some concerns that if addressed would improve the quality of the manuscript

**Major Compulsory Revisions**

1. It would provide a lot of support for the DMR assay, if the authors would take one or two DOR, MOR and KOR ligands and test P38 MAPK activation using a western blot analysis on HEK-MOR, HEK-DOR and HEK-KOR cells, to support their DMR findings using SB202190. I am aware that the authors already present a lot of data and figures, but this should not be too hard of an experiment to perform and would strengthen their findings.

2. The authors show that BNTX and beta-FNA have off-target effects as evident from their DMR responses in “empty” HEK cells, and exclude these ligands from biases agonism analysis, yet they keep using these compounds in their experiments (Figure 7-9). The off-target effects make it hard to draw any firm conclusions about the effects of these drugs in these experiments, and they should probably be excluded from these experiments.

3. The authors could discuss the possibility that, for example, PTX treatment unnaturally shifts receptor signaling to a different signal transduction pathway. As such the lack of a complete blockade of signal with PTX does not necessarily mean that under naïve conditions (when functional Gi is present) a ligand signals...
through Gi-independent pathways. Additionally have the authors tried to perform a dose response of PTX on “empty” and opioid receptor expressing HEK cells to determine if this causes a DMR?

Minor Essential Revisions

1. The heat maps presented in the manuscript figures have different maximum values. For instance 135pm in figure 4, 100pm in figure 5 and 45pm in figure 6. This makes it harder to compare whether for example the p38 MAPK inhibitor has less effect in HEK-DOR than HEK-KOR. For comparison it would be better to have identical “heat ranges”.

2. The results section contains a lot of information that is better fitted in either introduction, discussion or the method section (e.g. paragraph 4, page 5 and paragraph 3, page 6).

3. The authors do not clearly explain their rationale for using 64nM as the fixed concentration to study dose-dependent desensitization with DPDPE (Figure 7), dose-dependent inhibition with BRL-57532 (Figure 8) and DAMGO (Figure 9).

4. On page 10, the authors claim that one of their most notable findings is that responses in SH-SY5Y cells are not identical to solo activation of MOR in HEK-MOR, based on a DMR response for a DOR agonist in SH-SY5Y cells, but not HEK-MOR cells. This is not very surprising as SH-SY5Y cells do express some DORs, as the authors mention on page 5.

5. It’s hard for the reader to judge full and partial agonist responses based off the heat maps especially when comparing the weaker full agonist response in SH-SY5Y cells compared to the full agonist responses in HEK-MOR.

6. The reasoning behind some of the clustering that is shown in the figures is not easily to understand. In particular in figure 1. For example in figure 1B, why are DSLET and dynorphin A-18 not in the same cluster?

7. Table 2 appears to contain some errors. For example DPDPE has a literature classification as kappa agonist, and DAMGO as an opioid agonist. The authors are advised to double check their table.

8. The pink dots in Figure 3B and 3D appear to be identical, although the Y-axis is different. Most likely the pink dots in figure 3B are wrong, as it is hard to imagine why compounds with DOR affinity would displace DAMGO from SH-SY5Y cells equally well as DPDPE from HEK-DOR.

9. Why did the authors choose to show the raw DMR for the PTX treatment, the data would be clearer represented if the PTX reduction in DMR compared to vehicle treatment showed up in green in the figures.

10. The term false-colored heat map seems to be out of place as there were never any true-colors.

11. Page 15 in the middle “such as DPDPE who appear to act as full ligands”.

The authors probably mean agonists not ligands.

12. Bottom of page 15;"the other four agonists" The reader has to go back 2 sentences to assume that the fifth agonist is dynorphin A 1-13.

13. In figure legend 3 the authors write DMAGO instead of DAMGO.

Discretionary Revisions

1. Why is the expression level (Bmax) of the KOR in HEK-KOR cells unknown, it shouldn’t be too hard to obtain this data?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests