Author's response to reviews

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

Authors:

Megan Morse (morseme@gmail.com)
Haiyan Sun (haiyan.sun@gmail.com)
Elizabeth Tran (frane@corning.com)
Robert Levenson (rlevenson@hmc.psu.edu)
Ye Fang (fangy2@corning.com)

Version: 5 Date: 10 October 2012

Author's response to reviews: see over
October 10, 2012

Responses to reviewers’ comments

We highly appreciate the reviewers’ positive comments, and more importantly their instructive criticism which assists the quality improvement of our manuscript.

Reviewer: Richard M van Rijn

Reviewer's report:
Minor essential revision:
I am still somewhat puzzled by the pink dots in Figure 4B and 4D. The way I understand the figure each pink dot represents a value based on literature (not the current experiment) of a specific compounds affinity on DOR (X-axis) and the ability of the compound (corrected for concentration and its affinity) to displace either DAMGO from SH-SY5Y cells or DPDPE from HEK-DOR cells (Y-axis). The blue dots represent actual data obtained by the authors using the DMR assay.

Response: this is correct.

If this is correct, than I am surprised that for example a compound with the highest affinity for DOR (most left pink dot, naltrindole?) displaces both DAMGO (4B) and DPDPE (4D) by 100%.

Response: DAMGO binds to MOR with a Ki of 1.1nM, and to DOR with a Ki of 64.7nM. Thus, it is expected that the highly potent DOR antagonist naltrindole can block the DPDPE DMR in HEK-DOR cells (as shown in Fig.4D). For the same reason, if the DAMGO DMR in SH-SY5Y cells is due to the activation of DOR only, naltrindole should also completely block the DAMGO DMR. However, our data showed that naltrindole actually only blocked the DAMGO DMR by ~83% (Fig.4B). This suggests that the DAMGO DMR is mostly due to the activation of the MOR in SH-SY5Y cells.
And since the pink dots are identical for both graphs, this means that every compound supposedly displaces DAMGO from SH-SY5Y cells (expressing MOR and DOR) and DPDPE from HEK-DOR cells to the same extent. If I am incorrect in my assessment of the figure I hope the authors would be able to clarify the figure legend to avoid misinterpretation.

**Response:** The pink dots are indeed identical for both Fig.4B and D. For Fig.4B, we assumed that the DAMGO DMR in SH-SY5Y is originated from the activation of DOR only, in order to compare the actual results with the theoretically calculated inhibition profiles for different ligands acting on the DOR sites. Based on these comparative analyses, we know that the DAMGO DMR is mostly due to the MOR, but also activate DOR.

**Response:** to clarify, we revised as follows in Page 18 to 19: “To best illustrate this, we first assumed that the DAMGO DMR in SH-SY5Y cells is originated from the activation of MOR or DOR alone, and then compared the actual DAMGO response with the calculated one for each ligand based on its reported affinity for the MOR (Fig.4a) or DOR (Fig.4b), respectively. This analysis showed that three potent MOR antagonists, β-funaltrexamine, levallorphan and nor-binaltorphimine, appeared to be less potent to block the DAMGO-induced DMR in SH-SY5Y cells than that would be expected at MOR binding sites; conversely, three agonists including SKF10047, ICI 199,441 and DIPPA desensitized SH-SY5Y cells with greater potency than their reported affinities at the MOR, and the remaining ligands gave rise to expected results (Fig.4a). This suggests that the DAMGO response has additional signaling component beside the MOR. Further, the DOR-selective agonists including deltorphin II, SNC121, BUBUC, SNC80 and DPDPE desensitized SH-SY5Y cells with lower potency than that would be expected at DOR binding sites, but the rest ligands behaved as expected at DOR binding sites (Fig.4b), suggesting that the DAMGO response in SH-SY5Y cells has additional signaling component beside the DOR. As comparison, the DAMGO induced DMR in HEK-MOR cells after pretreatment with library ligands was correlated well with their known binding affinities, with an exception of a group of antagonists including nor-binaltorphimine, N-benzylatrindole, naloxone methiodide, naltrindole, and naltriben (Fig.4c). Similarly, the DPDPE-induced DMR in HEK-DOR cells after the ligand pretreatment was mostly correlated well with their known binding affinities, except for a group of opioid antagonists including naloxone HCl (Fig.4d).”

Otherwise, the authors have adequately addressed the concerns raised by me previously. Some figures (8-10) in the PDF did show up properly, although they were clear from the supplemented word file. Overall I believe the manuscript will be of interest to the scientific community and readership of BMC Clinical Pharmacology to warrant publication.
Response: Thanks for the positive comments.

Ye Fang, Ph.D
Research Fellow, Corning Inc