Agonist-Induced Endocytosis of the Mu-Opioid Receptor

Patrick McGarrah, Ping-Yee Law, Ph.D. (advisor)
Department of Pharmacology, University of Minnesota Medical School

Introduction

Opioids are the most potent analgesic medications available for the treatment of moderate to severe pain. However, their use is limited by the risk of tolerance development, addiction, and potentially fatal side-effects such as respiratory depression. With the development of tolerance, a patient requires progressively larger dosages in order to achieve the same initial analgesic effect.[1] An understanding of how opioids produce tolerance and dependence is essential to maximizing their clinical utility and reducing their addictive liability.

All clinically prescribed opioids activate the mu-opioid receptor (MOR), and it is believed that tolerance development involves changes in receptor function. The precise mechanism of these changes is unclear, but it likely involves desensitization, downregulation, and perhaps endocytosis. Whistler et al. have postulated that an agonist's ability to induce receptor endocytosis is directly related to its potential to produce tolerance and dependence.[3] However, other investigators have found conflicting data.[2] For example, Zhao et al. found that adenylyl cyclase superactivation, a hallmark of dependence, was unaffected when MOR internalization was inhibited.[4] Nevertheless, the fact that morphine does not promote receptor internalization, and whether this is related to its ability to produce tolerance, is still under investigation.

It has been proposed that delta-opioid receptors (DOR) can form heterodimers with MORs.[5] The DOR has previously been shown to be involved in morphine activity, but debate persists as to the precise role. The present study examines receptor internalization of co-expressed MORs and DORs after treatment with various ligands. Specifically, it will be shown that morphine can induce receptor internalization in HT-1080 cells when administered along with a delta-specific ligand (DPDPE).

Materials & Methods

Recombinant rat MOR and DOR genes were cloned into the pCDH lentiviral backbone under a CMV promoter. The receptor sequences were cloned adjacent to either GFP or mCherry to generate fluorescent fusion opioid receptors. These constructs were used to generate lentiviruses that could induce expression of MOR or DOR.

HT-180 cells were infected with MOR-mCherry and DOR-GFP viruses to achieve co-expression of MOR and DOR. Cells were visualized by confocal fluorescence microscopy before and after a 90 minute incubation with either saline, 1 μM etorphine, 1 μM DAMGO, 1 μM DPDPE, 10 μM morphine, or 10 μM morphine + 1 μM DPDPE.

Primary cortical neurons were isolated from postnatal day 1-2 rats and cultured until maturity at 21 days. Cultures were then infected with various MOR or DOR viruses and visualized 4 days post-infection.

Results

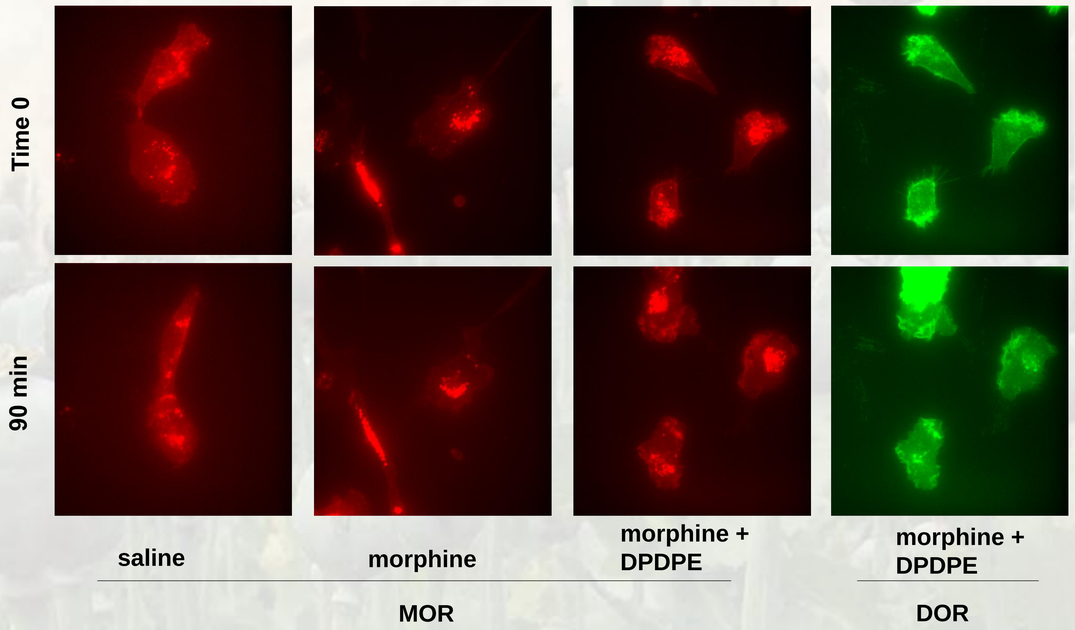

Figure 1. Confocal fluorescence microscopy of HT-1080 cells expressing MOR-mCherry (red) and DOR-GFP (green) fusion proteins. Little change in membrane or internal fluorescence is observed for either saline or morphine treatment. However, morphine + DPDPE treatment results in decreased membrane and increased internal fluorescence of both MOR and DOR, suggesting internalization of the receptor.

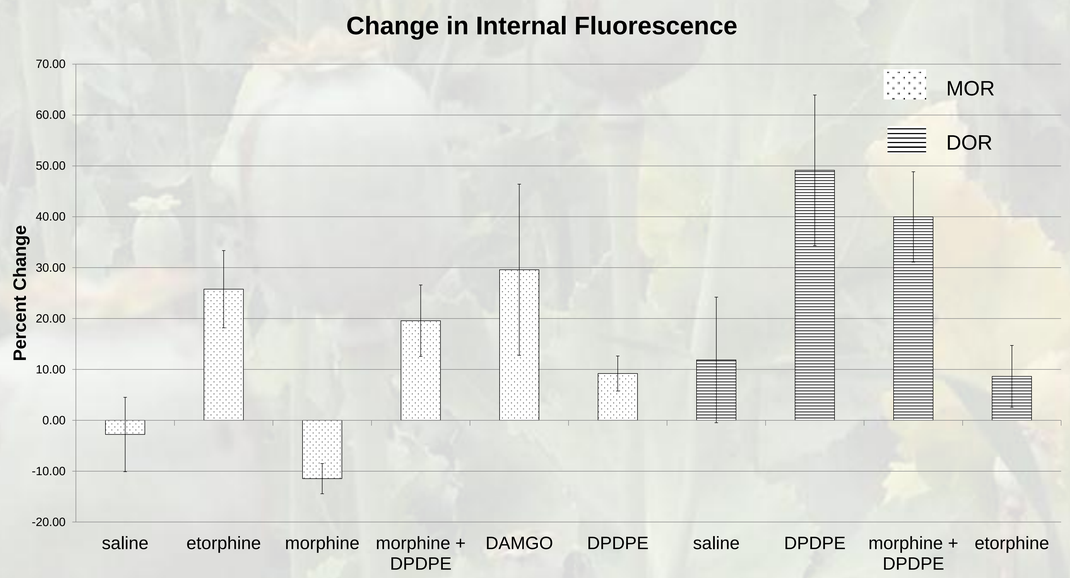

Figure 2. Percent change in internal fluorescence after 90 minute drug treatment. Morphine + DPDPE increased internal fluorescence significantly compared to morphine alone ($p<0.001$) and saline ($p<0.02$). Etorphine and DAMGO are both ligands known to produce internalization of MOR. Error bars ± 1 SE

Conclusion

Treatment of HT-180 cells infected with MOR and DOR fusion proteins with morphine + DPDPE appeared to cause internalization of MORs. Treatment with either morphine, saline, or DPDPE alone did not cause internalization of MORs. This suggests that activation of the delta opioid receptor may play a role in the endocytosis of the mu-receptor. It is possible that this occurs by activation of a mu-delta heterodimer, where the mu receptor must be activated in order to become endocytosed.

However, these findings need to be confirmed in larger sample sizes. In addition, the experiments need to be repeated in neuronal cells, which will more closely model the environment of the central nervous system. If MOR and DOR do in fact function as heterodimers, it opens the possibility of designing therapies targeted at this specific receptor complex, perhaps with equal analgesic potency but less tolerance than morphine.

References

1. Lin HY et al. (2012) *J Pharmacol Exp Ther.* Apr; 341(1): 115-125.
2. Law PY, Loh HH (2006) "Opioid receptor signaling and regulation." In *Handbook of Neurochemistry and Molecular Neurobiology.*
3. Whistler JL et al. (1999) *Neuron*. 23: 737-746.
4. Zhao H et al. (2006) *Mol Pharmacol.* Apr; 69(4):1421-1432.
5. Gupta A et al. (2010) *Sci Signal.* Jul 20;3(131):ra54.

Acknowledgements

I would like to thank Dr. Ping-Yee Law for his help on designing and executing these experiments. I would also like to thank David Lin for his help on producing lentiviruses, and Angel Kamm for her advice on primary culture.

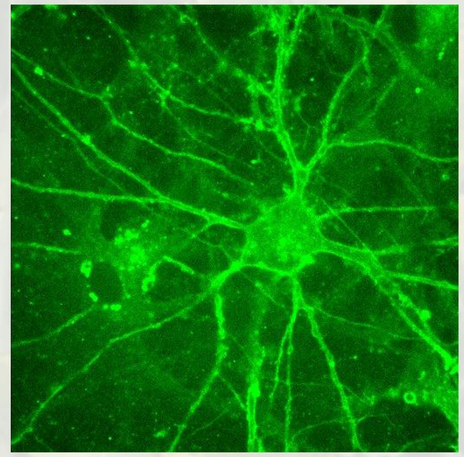

Figure 3. Cortical neuron showing membrane fluorescence from MOR-GFP