

Exogenous Ligand of GPR171 Reward Response in the Ventral Tegmental Area

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ABSTRACT

Opioids are commonly used pain medications, but also have an adversely high addiction rate. The G protein-coupled receptor GPR171, with its agonist MS15203 is shown to lessen chronic and acute pain and increase morphine analgesia acutely. This effective coupling utilizes a lower dose of the opioid, which in turn decreases chance of addiction. However, the possible addiction factor of GPR 171 was still unknown. The objective of this recent study has been to quantify the biochemical reward effects of GPR 171. Mice were treated with morphine and MS15203, both jointly and independently, and immunohistochemistry was used to find effects on the firing of dopamine neurons in the ventral tegmental area (VTA), a dopaminergic center that is key to opioid-induced reward. The immediate early gene c-Fos was used to indicate the neuronal activity of the dopamine neurons, with an increase of c-Fos showing more dopamine release, and higher feelings of reward. Methods are still in progress for this study, but evidence is indicating that MS15203 does not increase c-Fos activation when combined with morphine and is therefore less rewarding than opioids.

INTRODUCTION

Opioids are a widespread medication effective in reducing pain, but the consequence of addiction is pushing for new treatments. G proteins are commonly targeted in many types of medication and are a target of possible new pain treatments. GPR171, an inhibitory G protein, was recently orphaned, when its ligand BigLen was discovered in 2013. The Bobeck Lab in 2019 discovered that GPR171 is in the ventrolateral periaqueductal grey, a brain structure crucial in pain modulation. An exogenous agonist that binds to GPR171, MS15203, was then found. When MS15203 was injected into mice, it increased morphine antinociception (blocking pain stimulus through sensory neurons). This discovery could lead to a coupling medication, and a decrease dosage of the opioid. However, the reward effects of ligand MS15203 and receptor GPR171, were still unknown. Recently the Bobeck lab mapped GPR171 throughout reward structures in the brain and found evidence of GPR171 in reward structures of the mouse brain, including the VTA, which is dense with dopaminergic neurons and key to opioid reward. Under microscope, there is colocalization between tyrosine hydroxylase, a dopamine enzyme commonly used to represent dopamine neurons, and GPR171. This leads to the assumption of GPR171 receptors located on dopamine neurons. With this knowledge, we began interperitoneally injecting MS15203 into mice brains, both independent and coupled with morphine, and looking at the activity of dopamine neurons in the VTA. C-Fos, a protein released from neuronal activity, was used to measure reward activity in the VTA.

METHODS

Drug Injection and Perfusion

Male mice (n=16) were split into four groups, and interperitoneally injected with designated drugs: morphine (10 mg/kg), morphine (10mg/Kg) + MS15203 (10mg/Kg), MS15203 (10mg/Kg), and saline (.9%). 90 minutes later animals sacrificed with isoflurane, perfused with 4% paraformaldehyde, and stored refrigerated in PBS.

Immunohistochemistry

Stored brains were dissected to isolate the midbrain. Midbrains were sliced with a vibratome at 50 microns, and stored in 1x PBS in refrigeration. Immunohistochemistry was performed on the slices, which started with 30 min in 1% sodium borohydride, then one hour in 5% normal goat serum blocking buffer. Then a refrigerated overnight of 2 primary antibodies, tyrosine hydroxylase (TH, mouse, Invitrogen), and early gene c-Fos (rabbit, Abcam), in a 1:500 dilution. Last, for five hours an incubation of secondary antibodies capable of fluorescent staining of was performed, with the antibodies goat anti-mouse (488, Invitrogen) and goat anti-rabbit (594 Invitrogen). Finished slices were positioned on glass microscope slides, using mounting media.

Microscopy and Statistical Analysis

A Keyence microscope was used to image the fluorescently stained VTA. Green coloring represents TH, indicating location of dopamine neurons, while red coloring shows c-Fos activated cells, indicating recently fired neurons.

Images of C-fos dopamine cells were processed with BZ-X800, to yield highest quality images for counting. C-fos dopamine cells were hand counted by a blind experimenter, Emmaline Haderlie. The average number of C-fos cells of four groups (morphine, morph + MS15203, MS15203, and saline) were statistically analyzed, with ANOVA and holm-sidak multiple comparison tests. Results were concluded.

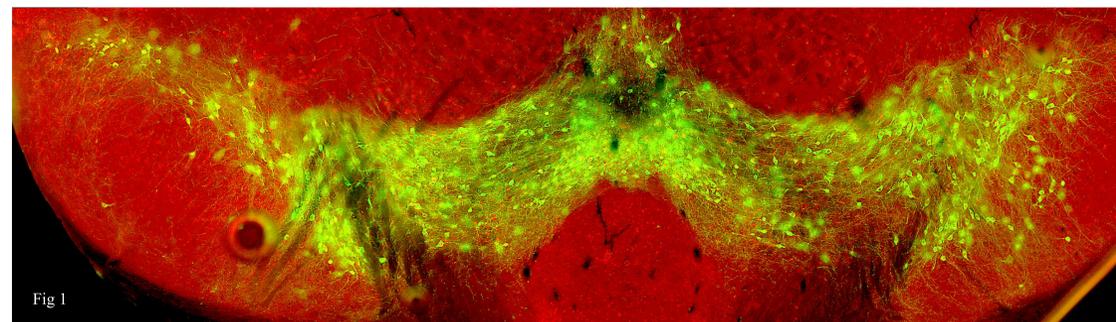


Fig 1

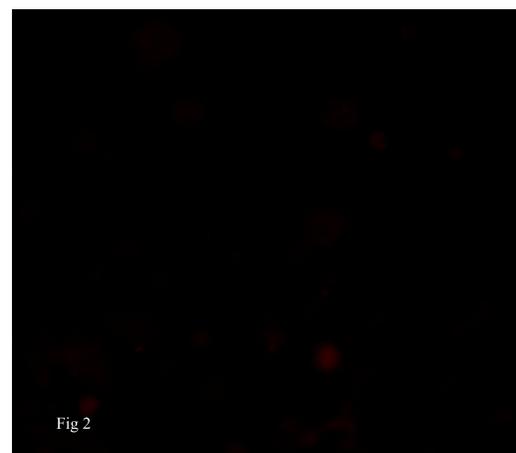


Fig 2

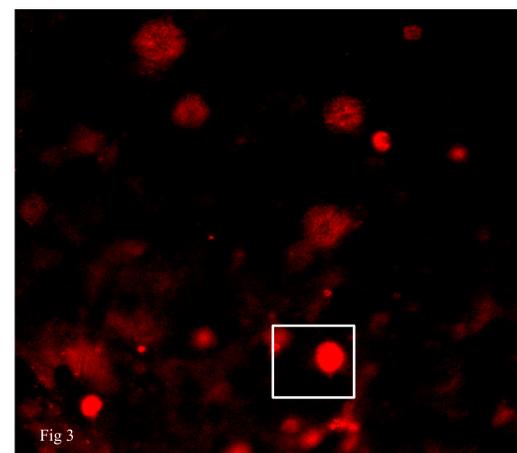
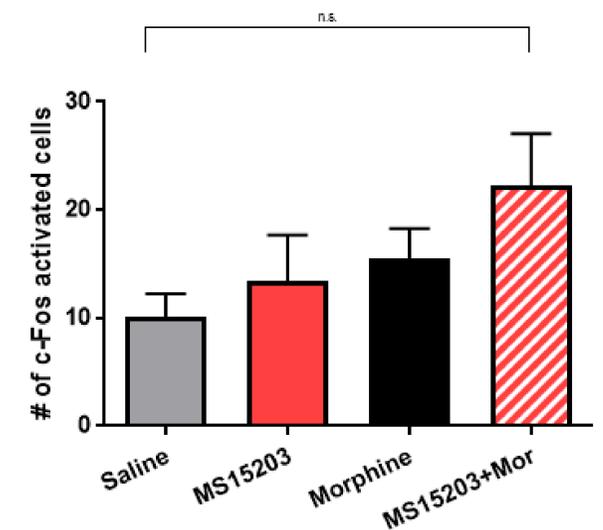


Fig 3

c-Fos activation in the VTA



CONCLUDING DISCUSSION

No concrete results can be given at this time, as methods are still being conducted, with studies currently in the statistical analysis stage. However, this experimental design is a secondary round, with the identical methods performed earlier this year on 12 mice. Results from this primary experiment showed a trend of c-Fos with morphine+MS15203 > morphine > MS15203 > Saline. This trend indicates that MS15203 does increase c-Fos in the VTA, and therefore reward, as the morphine + MS15203 has the most neuronal activity. But the trend shows individual MS15203 elicits less reward than morphine, which is an exciting discovery. However, until statistical analysis is finished for this secondary round of experimentation, no statistical significance, can be decided.

Fig. 1 was taken under microscope 11/12/21 in lab, at the ventral tegmental area (VTA) location. The relationship between c-Fos activated neurons (red) and TH VTA neurons (green) is the focus.

Fig. 2 shows left side of VTA in Mou 4 before image processing with BZ-X800.

Fig. 3 shows left side of VTA in Mou 4 after image processing. The white box outlines an example neuron counted in the microscopy methods as evidence of dopamine release.

Fig. 4 are the results from the primary round of experimentation referenced in the conclusion. Results in the secondary round of methods have not been reached yet.



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