Mapping a novel G-protein coupled receptor to understand its role in morphine reward

Nick Beus, Max McDermott, Dr. Erin Bobeck, Utah State University, Department of Biology

Introduction

Due to the addictive nature of opioid drugs, those exposed to opioid treatments over long periods of time are at high risk of abusing their medication and possibly overdosing. Cleary, alternative pain therapeutics are needed. Research in our lab has shown that a new G-protein coupled receptor enhances the pain relieving properties of morphine (McDermott, *et al.* 2019). This suggests its possibility in enhancing pain relief while necessitating a lower dose of morphine. In order to understand this novel GPCR's mode of action and its actions *in vivo*, molecular and behavioral actions must be evaluated. Behavioral research in our lab has shown that an agonist for GPR171 is not rewarding. (Figure 1) In this experiment we map GPR171's throughout the reward pathway to further our understanding of this novel receptor's role in reward and morphine reward.

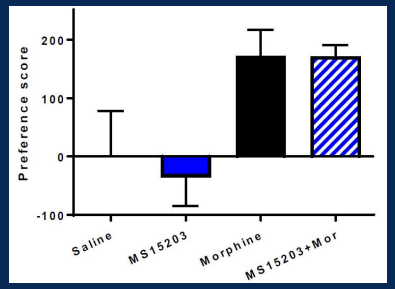

Figure 1: Conditioned Place Preference: Time spent in drug pared chamber show that the novel GPCR agonist, MS15203, is significant is not significant from saline and therefore is not rewarding. MS15203+Mor is not more rewarding that morphine alone. **p<.005

Approach

Key areas of the reward pathway (Figure 2): the prefrontal cortex, the hippocampus, nucleus accumbens, and the basolateral amygdala were evaluated. Immunohistochemistry was done to discern the location and density of GPR171 in these brain structures. Immunohistochemistry (IHC) is done using fluorescent antibodies specific to specific epitopes present on biological structures, such as G-protein receptors. In this assay we utilize IHC and a previously vetted antibody specific to GPR171 (rabbit, 1:400; Genetex, Irvine, CA) (Bobeck et al., 2017) to fluorescently mark GPR171 receptors in reward structures. A Keyence microscope was then used to visualize the expression of fluorescently marked GPR171.

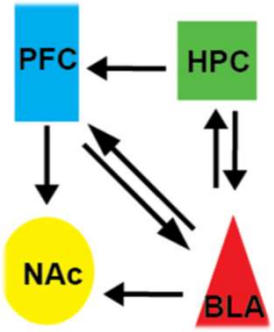

Figure 2: The reward pathway

GPR171 in the reward pathway

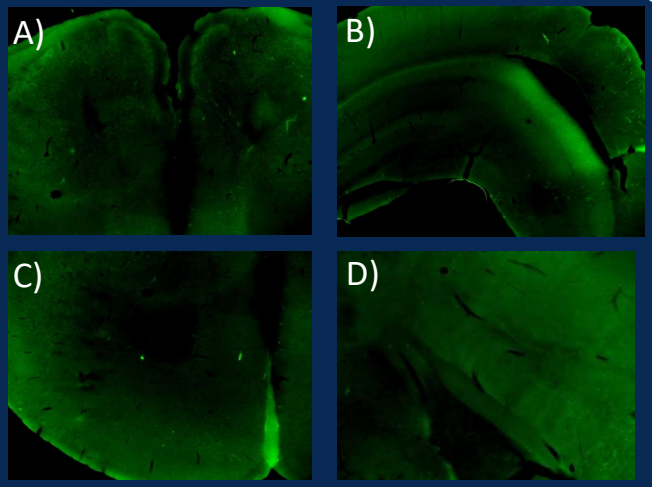

Figure 3: 4x Magnification of IHC stain of GPR171 in reward structures of the brain using fluorescent microscopy.
Green punctates shows IHC staining where GPR171 is present.
A) Prefrontal Cortex (High expression of GPR171). B) Hippocampus. C) Nucleus Accumbens. D) Amygdala.

GPR171 at high magnification

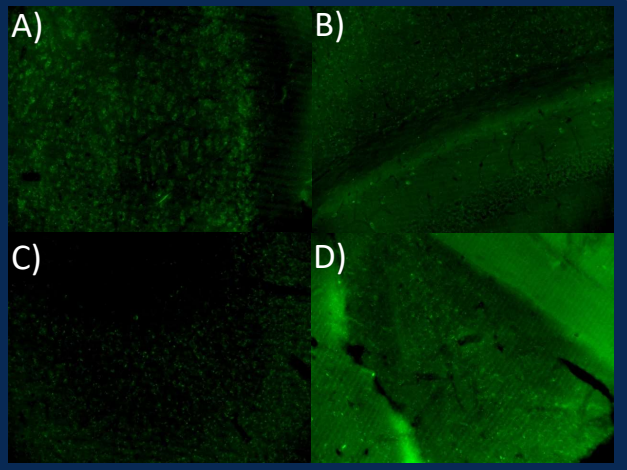

Figure 4: High magnification of IHC stain done on reward structures of the brain using fluorescent microscopy.
Green stain shows IHC stained area where GPR171 is present.
A) Prefrontal Cortex (High Expression of GPR171).
B) Hippocampus
C) Nucleus Accumbens
D) Amygdala

Conclusions

GPR171is present on neurons in key reward structures of the brain (Figure 3, Figure 4). Though there is shared presence of mu opioid receptors and GPR171 in the hippocampus, prefrontal cortex, amygdala, and nucleus accumbens, (DiFeliceantio & Berridge, 2012; Hioki, et al., 2020; van Steenbergan, Elkemo, & Leknes, 2019; Steffenson, et al., 2006) interaction between GPR171 and the mu opioid receptors is not seen behaviorally as in pain (McDermott et al. 2019). Behavioral studies suggest that an agonist does not decrease morphine conditioned place preference, however, future studies need to be done to investigate the potential colocalization and modulatory activity these receptors may have on one another in regard to reward.

Study conducted with funding from a USU Undergraduate Research and Creative Opportunity Grant and lab assistance from the USU Department of Biology.

Nick Beus
Utah State University
Neurobiology
nick.beus@aggiemail.usu.edu