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Engineering Bioluminescent Sensors of Cyclic AMP to Study Opioid Signaling

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ABSTRACT

Opioids are small signaling molecules which bind to opioid receptors on the surface of cells. The kappa opioid receptor (KOR) is one of three major types of opioid receptors found in human neurons. When an opioid binds to a KOR, a variety of biochemical signaling pathways are activated inside the cell. Each of these pathways are associated with different physiological effects of KOR activation. The production of a small signaling molecule, cyclic adenosine monophosphate (cAMP), is known to be inhibited during KOR activation of the analgesic (pain-killing) signaling pathway. The ability to interrogate the individual responses of KOR signaling pathways in a living mammal would greatly improve our understanding of how opioids work in the brain. To this end, we have developed a biosensor functioning via bioluminescent resonance energy transfer (BRET) as a tool for both fluorescent and luminescent ratiometric quantification of cAMP. We couple two fluorescent proteins, emitting at different wavelengths, to a luciferase which provides chemiluminescent excitation energy for the complex. The intensity of the two emitted wavelengths vary inversely to each other in response to the presence of cAMP. Calculating the ratio of the two emission intensities creates a metric for cAMP concentration that is normalized to the concentration of our sensor, allowing quantitative comparison across trials. The application of our sensor for dual-color live-cell microscopy was demonstrated in mammalian cells using fluorescence and bioluminescence microscopy. Further proof-of-principle studies in KOR-expressing mammalian cells demonstrates the viability of our sensor for live-cell KOR signaling.

KEYWORDS

fluorescent protein, BRET, live-cell imaging, cAMP, kappa opioid receptor, bioluminescence