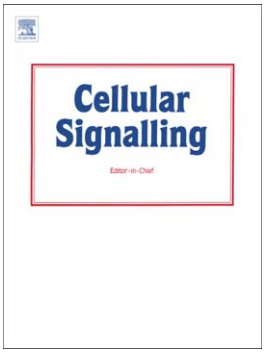

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Heterodimerization of human orexin receptor 1 and kappa opioid receptor promotes protein kinase A/cAMP-response element binding protein signaling via a Gαs-mediated mechanism*

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*Running title: OXR1/KOR dimerization increases PKA/CREB activity

Key words: G-protein-coupled receptor (GPCR), heterodimerization, orexin (hypocretin) receptor 1 (OX1R), kappa opioid receptor (KOR), bioluminescence resonance energy transfer (BRET), Förster resonance energy transfer (FRET)

ABSTRACT

Orexin and dynorphin are co-expressed in the same synaptic vesicles of hypothalamic neurons and play opposing roles in cocaine self-administration, brain stimulation reward, and impulsivity in ventral tegmental area (VTA), where dopamine neurons express both OX1R and KORs. However, detailed mechanisms of how the coreleased peptides and both receptors fine-tune their signalings and physiological/behavioral effects together remain unclear. Here we explore the possibility of heterodimerization between OX1R and KOR and reveal novel signal transduction mechanisms. First, we demonstrated co-expression of OX1R and KOR in rat hippocampal neurons by single-cell PCR. Furthermore, heterodimerization between OX1R and KOR was examined using bioluminescence and Fluorescence resonance energy transfer (BRET and FRET). Our data revealed that human OX1R and KOR heterodimerize, and this heterodimer associates with Gαs, leading to increased protein kinase A (PKA) signaling pathway activity, including upregulation of intracellular cAMP levels and cAMP-response element (CRE) luciferase reporter activity, resulting in increased cAMP-response element binding protein (CREB) phosphorylation. These results support the view that OX1R and KOR heterodimerization might have an anti-depressive role.

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Abbreviations : GPCR, G-protein-coupled receptor; OX1R, orexin (hypocretin) receptor 1; KOR kappa opioid receptor; BRET, bioluminescence resonance energy transfer; FRET, Fluorescence resonance energy transfer; CREB, cAMP-response element binding protein; PKA, protein kinase A; CUMS, chronic unpredictable mildstress; Rluc, Renilla reniformis luciferase; YFP, yellow fluorescent protein; CFP, cyan fluorescent protein; ELISA, Enzyme-Linked Immunosorbent Assay