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Alternatively spliced mu opioid receptor intracellular C-termini encoded by exon 7 are important for fentanyl actions

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Background: One type of alternatively spliced Oprm1 variants is full-length 7TM C-terminal variants. The roles of these 7TM C-terminal variants have been demonstrated in the C-terminal truncation mouse models. Particularly, exon 7-associated C-terminal truncation in C57BL/6J mice (mE7M-B6) diminished morphine tolerance and reward without altering physical dependence, like those seen in a β-arrestin2 KO mouse. Together with the results from cell-based studies, it suggests a physical and functional interaction of E7-associated C-terminal tails with β-arrestin2 that contributes to morphine desensitization and tolerance.

Rationale/Significance: Further investigating the role of E7-associated C-terminal tails in fentanyl actions will advance our understanding of complex mu opioid pharmacology.

Hypothesis: E7-associated C-terminal tails play important and distinct roles In opioid pharmacology.

Result: In mE7M-B6 mice, fentanyl analgesia was not affected. However, fentanyl tolerance was significantly reduced, while fentanyl-induced physical dependence was slightly increased. Fentanyl reward determined by CPP was significantly reduced in mE7M-B6 mice. The mutations in the predicted phosphorylation sites of E7 diminished mu agonist-induced β-arrestin2 binding by mMOR-1O measured by NanoBit β-arrestin2 recruitment assay in OPRM1-knockdown Be(2)C cells. Mu agonist-induced receptor-protein interactions for E7 variants have also been investigated by using APEX2-TMT proteomics approach.

Discussion: Our results demonstrated that E7-associated C-terminal tails are important for fentanyl tolerance and reward, similar to what we observed for morphine actions. Mutagenesis study suggest that E7 contains specific phosphorylation sites in response to mu agonists for β-arrestin2 binding. These data further support the functional relevance of E7-associated splice variants in opioid pharmacology.

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