Examining the mu-opioid receptor bias of fentanyl and 2-benzylbenzimidazole opioids in clandestine drug markets

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Many pain medications act as agonists at the μ-opioid receptor (MOR). Unfortunately, MOR agonists can induce severe adverse effects. The development and misuse of novel synthetic opioids (NSO), many of which are highly selective MOR agonists, have resulted in serious intoxications and fatal overdoses. The newly emerging nitazene analogs appear to be more potent than fentanyl analogs, posing significant public health risks. As a G-protein coupled receptor, MOR can activate both Gi-proteins and β-arrestins to trigger downstream signaling cascades. Previous studies proposed that Gi-protein biased MOR agonists are safer than β-arrestin biased agonists. However, other studies argue that the reduced side effects of MOR agonists could be caused by low intrinsic efficacy. In this study, to rigorously characterize the bias profiles of NSOs, we carried out in vitro functional measurements of both fentanyl and nitazene analogs at MOR by applying the depletion approach, which involves depletion of MOR using the irreversible antagonist methocinnamox. By fitting the data to the operational model, a general mathematical model that explicitly describes agonism, the results from the cAMP inhibition and β-arrestin2 recruitment assays allow us to estimate the intrinsic efficacy with τ and to measure the intrinsic affinity (i.e., Kₐ) for each compound in the Gi protein and β-arrestin2 pathways. Overall, fentanyl and nitazenes demonstrated noticeably different pharmacological properties. The calculation of the “bias factor” shows that fentanyl is biased toward the Gi-protein pathway, while valerylfentanyl demonstrates some β-arrestin2 bias. Among the nitazene analogs, N-desethyl isotonitazene and isotonitazene are more potent than DAMGO in both the Gi protein and β-arrestin2 assays, but none of the nitazenes shows robust functional bias. Taken together, NSOs tested in this study show somewhat different bias profiles even within the same series of analogs, suggesting that the in vitro functional bias alone may not be adequate in evaluating the toxicity and abuse liability for a given opioid. Thus, a combined interpretation of the results from both in vitro and in vivo studies is required to fully understand NSOs.

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