American College of Neuropsychopharmacology

RESEARCH HIGHLIGHT

Opioid overdose and tolerance: is the recruitment of β-arrestin to the μ-receptor involved?

Alexander Gillis[1] and Macdonald J. Christie[1]✉

Neuropsychopharmacology (2021) 46:2226–2227; https://doi.org/10.1038/s41386-021-01121-3

The search for safer opioid analgesics that have reduced liability for tolerance, addiction, and lethal overdose due to respiratory depression has met with little success for well over a century. One of the more recent iterations of this pursuit has been the G protein signalling bias hypothesis of safer opioids. This posits that opioid agonists which fail to recruit β-arrestins to the μ-opioid receptor (MOR) will not produce the severe side effects of opioid-induced respiratory depression (OIRD), tolerance, or addiction because the recruitment of β-arrestins during receptor activation triggers signalling cascades that mediate these adverse effects. This idea has led to the description of multiple putatively G protein-biased MOR agonists, including a drug now approved by the FDA, oliceridine. The hypothesis was proposed following a prominent series of studies beginning two decades ago, that appeared to show that germline deletion of β-arrestin2 (also known as arrestin-3) in a mutant mouse greatly reduced morphine-induced OIRD [1], analgesic tolerance and other adverse effects (see [2]). However, more recent studies in at least four independent laboratories have failed to reproduce the original observation of blunted OIRD in the β-arrestin2 knockout mice [3, 4]. Studies in knock-in mice expressing a phosphorylation-deficient mutant MOR that does not recruit β-arrestins, effectively a G protein-biased MOR mouse, have similarly failed to find any reduction in OIRD when β-arrestins are not recruited during MOR activation (see ref. [2]).

In this issue of *Neuropsychopharmacology*, He et al. [5] replicate this negative result for OIRD and, importantly, provide a plausible explanation for the now unsubstantiated original findings. The problem appears to be that the initial experiments were performed on β-arrestin2 knockout mice with a mixed strain background, the colony being formed from the interbreeding of knockout 129 male mice with wild-type C57BL/6 females. He et al. [5] now report substantial differences in respiration between 129/SvJ and C57BL/6J inbred mouse strains and, critically, a resistance to morphine-induced respiratory depression in 129/SvJ mice. As the mice used in the original studies remained on a mixed 129-C57BL/6 background, the results may have been confounded by paternal 129 strain characteristics. This issue has long been known to potentially yield false positives in situations where genetically modified mice are derived by the inter-crossing of laboratory mouse strains, followed by limited backcrossing. By contrast, the more recent negative studies have uniformly been performed on a stable, congenic C57BL/6J background, following backcrossing in several independent laboratories for at least ten generations, eliminating this potential confound.

He et al. [5] then go further to challenge the notion that β-arrestin2 signalling in some way mediates OIRD using a 'recycling MOR' (RMOR) mutant mouse model the group previously developed. This mouse expresses a MOR/δ-opioid (DOR) chimeric receptor, in which the C-terminal region of MOR has been swapped with that of the DOR. He et al. had previously established that morphine much more effectively recruits β-arrestin2 to the mutant RMOR than in the wild type MOR. They argue that if β-arrestin2 signalling indeed mediates OIRD, then morphine-induced OIRD should be more severe in the RMOR mouse. Equi-analgesic doses of morphine, used for comparison as morphine is more potent in producing analgesia in RMOR mice, did not produce worse OIRD than in wild-type mice, evidence that enhancing recruitment of β-arrestin2 does not produce enhanced adverse effects. This result further contradicts what would be suggested by the G-protein bias hypothesis. It should be noted that the use of the RMOR mouse could be confounded by potential differences in receptor signalling due to the mutations and other, unknown receptor dynamic mechanisms potentially arising from the chimeric DOR C-terminal region, thus the interpretation of this result is not completely certain.

Further experiments by He et al. [5] suggest that the ability of different opioids to recruit β-arrestin2 is not correlated with the extent of OIRD (Figs. 3 and 5). Interpretation of the poor correlation is, however, limited by their use of single doses to measure OIRD, with only buprenorphine showing less peak OIRD, consistent with other studies [2]. Our previous studies and those of others, including with opioids considered by some to be G protein-biased, suggest that severity of OIRD is strongly correlated with the pathway independent intrinsic efficacy of MOR agonists, rather than their ability to recruit β-arrestins per se [2]. Together with the empirical evidence that any low intrinsic efficacy opioid agonist that is unbiased consistently yields very weak β-arrestin recruitment, the apparently improved safety of some opioids, including those purported to be G protein-biased, does not support the G protein-biased hypothesis [2]. It should also be noted that G protein signalling in respiratory network neurons, not the recruitment of β-arrestins, is the only clearly established mechanism of OIRD [2]. Indeed, Bachmutsky et al. [4] showed that MOR agonist-induced inhibition of respiratory control neurons persists in β-arrestin2 knockout mice, again in contrast to the G protein-biased agonist hypothesis.

He et al. [5] additionally aimed to understand the role of β-arrestins in the development of opioid tolerance. They observed

[1]School of Medical Sciences, The University of Sydney, Sydney, NSW, Australia. ✉email: mac.christie@sydney.edu.au

Received: 15 July 2021 Accepted: 20 July 2021
Published online: 16 August 2021

no effect of β-arrestin2 knockout on either the analgesic potency of morphine or the development of analgesic tolerance. This is at odds with the first studies on β-arrestin2 knockout mice. Previous studies have shown substantial differences in opioid tolerance between C57BL/6 and 129 strains [2], again suggesting a potential strain contamination confound in the original work.

Conversely, by employing the RMOR mouse that more efficiently recruits β-arrestins, He et al. [5] showed a dramatic reduction in the development of tolerance to morphine. This was interpreted as evidence that β-arrestin2 recruitment actually prevents, rather than facilitating, tolerance to opioid-induced effects. This is a long-standing hypothesis of the authors and colleagues that is diametrically opposed to the G protein-biased opioid hypothesis for improved opioid profile. He et al. [5] further show an apparent inverse correlation between analgesic tolerance and efficacy of opioids to recruit β-arrestin2. Some of their observations using different opioids, however, are not consistent with the literature. Chronic administration of fentanyl produces profound tolerance in other mouse studies [2], as both it and methadone do in patients. The authors' approach, employing equi-analgesic doses chronically, cannot overcome the well-known and very substantial pharmacokinetic, and intrinsic efficacy differences among the drugs tested. Recognising insurmountable differences in behaviour of drugs in the body, other investigators have tried to minimise complications by using constant infusion systems such as osmotic minipumps, which should minimise pharmacokinetic differences.

He et al. [5] interpreted the greater tolerance produced by chronic buprenorphine and oliceridine when compared to methadone and fentanyl treatment as reflecting differences in neuronal tolerance, as predicted by their hypothesis. Alternatively, the present work did not exclude an established effect whereby reduced intrinsic efficacy (e.g. buprenorphine) for G protein activation induces greater shifts in dose-response curves than for highly efficacious agonists (methadone, fentanyl) for the same level of system tolerance.

Independently of the potential for reducing OIRD and thus opioid overdose death, which is now largely understood to be independent of β-arrestin2 recruitment, it remains an intriguing open question whether or not opioids that more effectively recruit β-arrestins will actually produce less or more opioid tolerance, dependence, and addiction. The study of He et al. [5] has made a strong step in this direction, but only better controlled chronic opioid experiments will further inform new drug development.

REFERENCES

1. Raehal KM, Walker JK, Bohn LM. Morphine side effects in beta-arrestin 2 knockout mice. J Pharmacol Exp Therapeutics. 2005;314:1195–201.
2. Gillis A, Kliewer A, Kelly E, Henderson G, Christie MJ, Schulz S, et al. Critical assessment of G Protein-biased agonism at the μ-opioid receptor. Trends Pharmacol Sci. 2020;41:947–59.
3. Kliewer A, Gillis A, Hill R, Schmiedel F, Bailey C, Kelly E, et al. Morphine-induced respiratory depression is independent of β-arrestin2 signalling. Br J Pharmacol. 2020;177:2923–31.
4. Bachmutsky I, Wei XP, Durand A, Yackle K. ß-arrestin 2 germline knockout does not attenuate opioid respiratory depression. Elife. 2021;10:e62552.
5. He, L, Gooding, SW, Lewis, E, Felth, LC, Gaur, A, Whistler, JL. Pharmacological and genetic manipulations at the μ-opioid receptor reveal arrestin-3 engagement limits analgesic tolerance and does not exacerbate respiratory depression in mice. Neuropsychopharmacology. 2021. https://doi.org/10.1038/s41386-021-01054-x.

AUTHOR CONTRIBUTIONS

AG wrote the manuscript and MJC edited the manuscript.

FUNDING

Research of both AG and MJC is supported by NIH: R01DA048879-01 (Sub-award No. 2019-728-01) and National Health and Medical Research Council of Australia Ideas Grant APP2004671.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to M.J.C.