TRV130 and PZM21, Developed as Biased Opioid Analgesics With Fewer Side Effects, Have Incomplete Efficacy as μ Receptor Agonists

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Introduction

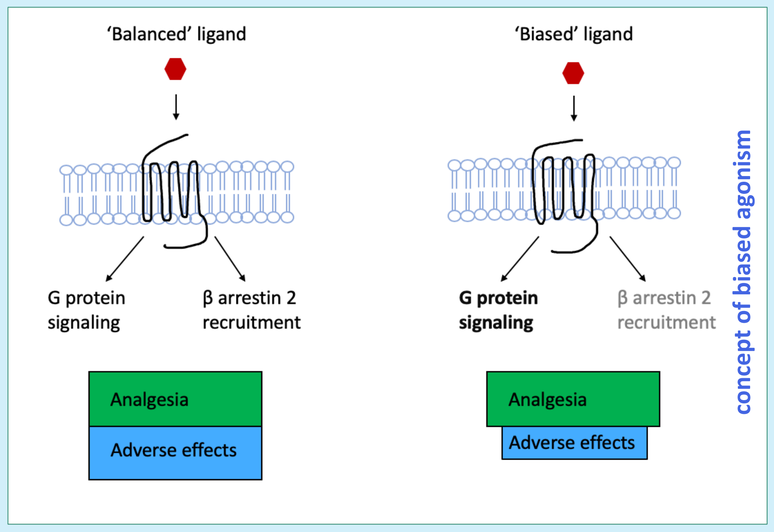
μ-opioid receptor (MOPR) agonists such as morphine are effective in acute, severe pain; however, their use in chronic pain is limited by side effects such as constipation, respiratory depression and tolerance.

Opioids are thought to produce analgesia via G-protein activation and adverse effects through recruitment of β arrestin 2 (βarr2)[1,2].

G-protein signaling → analgesia
βarr2 recruitment → side effects

'Biased agonism' describes the ability of an agonist to selectively activate one cellular pathway over another and can be estimated using efficacy and potency values of an agonist for each pathway[3]. Theoretically, this allows for the development of MOPR agonists which activate G-protein signalling, leading to analgesia, but do not recruit βarr2 and therefore lack side effects.

Recently, drugs biased towards G-protein activation have reportedly been developed. TRV130 and PZM21 are said to be maximally efficacious inhibitors of cAMP without recruiting βarr2, however, there is some controversy surrounding this; receptor overexpression in recombinant systems may influence the appearance of agonist efficacy, potency and therefore bias[4-6]. Are TRV130 and PZM21 truly biased, or is their inability to recruit βarr2 due to partial efficacy?



Aim

We aimed to determine whether recently developed compounds TRV130 and PZM21 are truly biased, by examining the efficacies of these MOPR agonists with full or partial MOPR availability

Methods

Cell culture and transfection: Chinese hamster ovary cells stably co-overexpressing human MOPRs and βarr2 were used for all experiments (DiscoverX). GloSensor-22F cDNA was transiently expressed by transfection using lipofectamine 2000 (Promega).

βarr2 assay: This assay uses enzyme fragment complementation technology to determine the level of βarr2 recruitment to MOPRs upon agonist binding.

cAMP assay: This assay uses biosensor technology and a conformationally modified enzyme to measure cAMP levels.

Reducing available receptor number: MOPRs were irreversibly blocked using the naltrexone derivative β-fulnaltrexamine (βFNA).

Data and statistical analysis: Data were normalised to maximum DAMGO response (βarr2 recruitment) or well-to-well maximum forskolin stimulated control (cAMP inhibition). Concentration-response relationships were fitted using a logistic function. Potency and efficacy were measured as log EC_{50}/IC_{50} and span of the relevant concentration-response curves, respectively. Statistical tests used were Student's t-tests (unpaired), one-way ANOVA with Bonferroni post hoc. $p < 0.05$ was considered statistically significant and represented as *.

Results

DAMGO, morphine, TRV130 and PZM21 have similar efficacies and potencies for cAMP inhibition

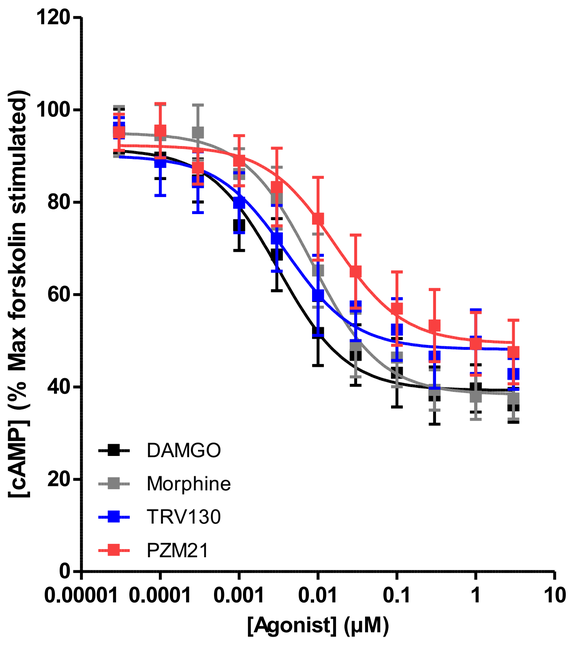


Figure 1 – MOPR agonist inhibition of cAMP
Suppression of cAMP by DAMGO (n = 11), morphine (n = 11), TRV130 (n = 8) and PZM21 (n = 8). There were no significant differences in their efficacies or potencies

Only DAMGO and morphine recruited βarr2

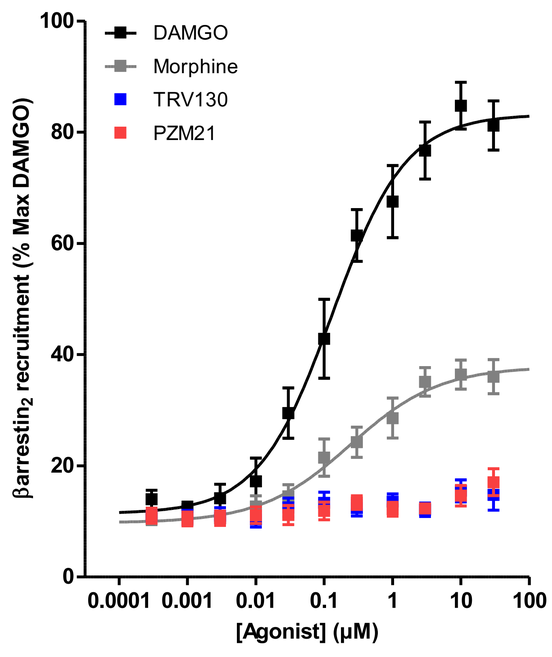


Figure 2 – MOPR agonist recruitment of βarr2
βarr2 recruitment by DAMGO (n = 16), morphine (n = 16), TRV130 (n = 12) and PZM21 (n = 12). TRV130 and PZM21 failed to recruit βarr2. DAMGO is significantly more efficacious at βarr2 recruitment than morphine. There were no significant difference in their potencies.

More available receptors are required for DAMGO to fully recruit βarr2

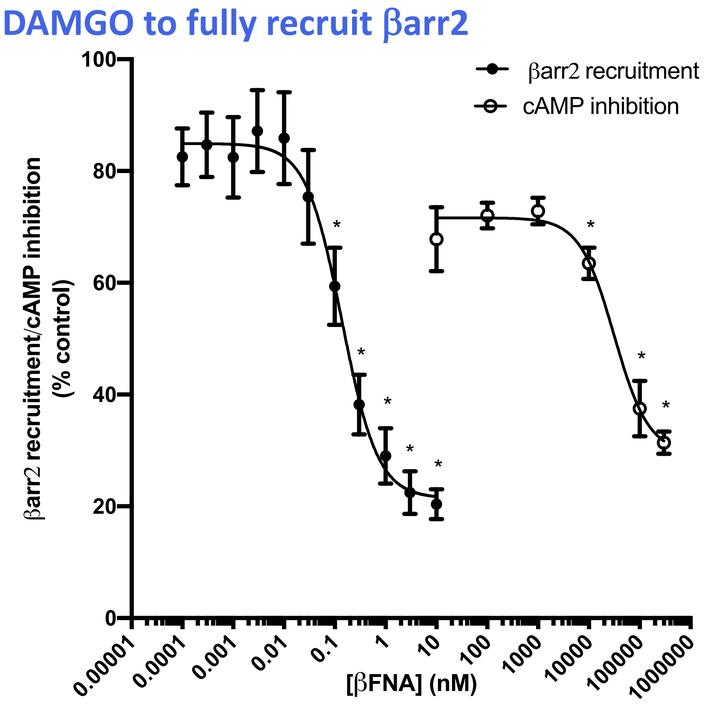


Figure 3 – βFNA concentration-response
DAMGO βarr2 recruitment (n = 4) and cAMP inhibition (n = 6). 0.1 nM βFNA significantly impaired DAMGO-induced βarr2 recruitment. 10,000 nM βFNA significantly impaired DAMGO-induced cAMP inhibition.

Morphine, TRV130 and PZM21 become partial agonists when the number of available receptors is reduced

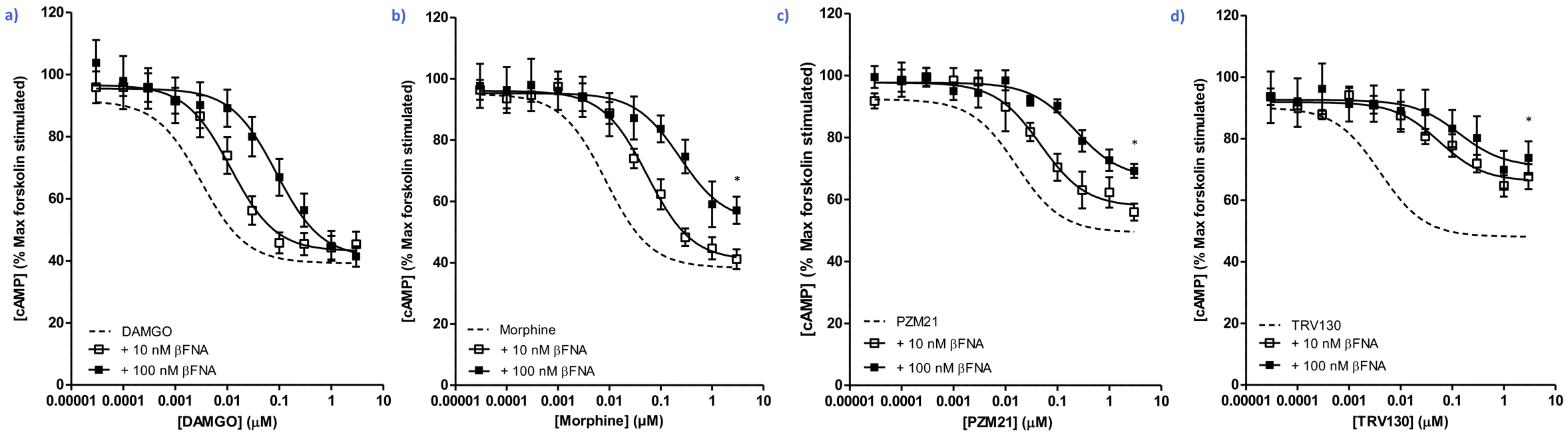


Figure 4 – The effect of reducing available MOPR number on cAMP inhibition
a) DAMGO (n = 11) efficacy for cAMP inhibition is unchanged when fewer receptors are available **b – d)** Morphine (n = 11), TRV130 (n = 8) and PZM21 (n = 8) have reduced efficacy for cAMP inhibition when fewer receptors are available

Conclusions

- In the presence of spare receptors, TRV130, PZM21 and morphine appear biased towards G-protein activation
- When the number of available MOPRs is reduced, the efficacies of these agonists for cAMP inhibition are markedly reduced
- These data are consistent with the idea that TRV130 and PZM21 are partial agonists
- Recombinant systems overexpressing receptors, in which bias is generally determined, may lead to false identification of bias
- **Relevance for patient care**: despite possibly having fewer side effects than morphine, TRV130 and PZM21 are likely to be weaker analgesics, thus compromising analgesia and hindering the search for novel analgesics which are effective without causing adverse effects.

References

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