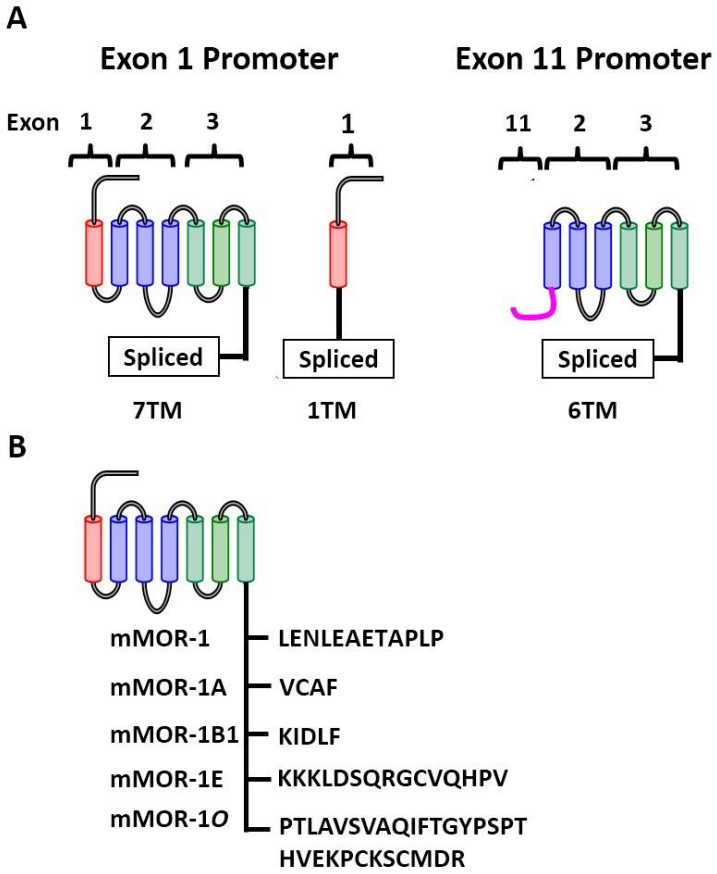
Pharmacological characterization of levorphanol, a G-protein biased opioid analgesic

Le Rouzic et al.

Supplement

Fig. 1: Schematic of *Oprm1* gene products



a) Classes of *Oprm1* gene splice variants: The *Oprm1* gene is comprised of over a dozen exons under the control of two distinct promoters, one associated with exon 1 and a second with exon 11. The exon 1 promoter generates the full length, 7 transmembrane (7TM) and single TM (1TM) variants while the exon 11 promoter yields the 6TM ones. All three classes undergo alternative splicing downstream of exon 3 with various combinations of exons that yield different amino acid sequences.

b) C-terminal sequences of select 7TM variants: Amino acid sequences downstream of exon 3 are presented for the five splice variants used to assess function. Note the differences in length and putative phosphorylation sites.

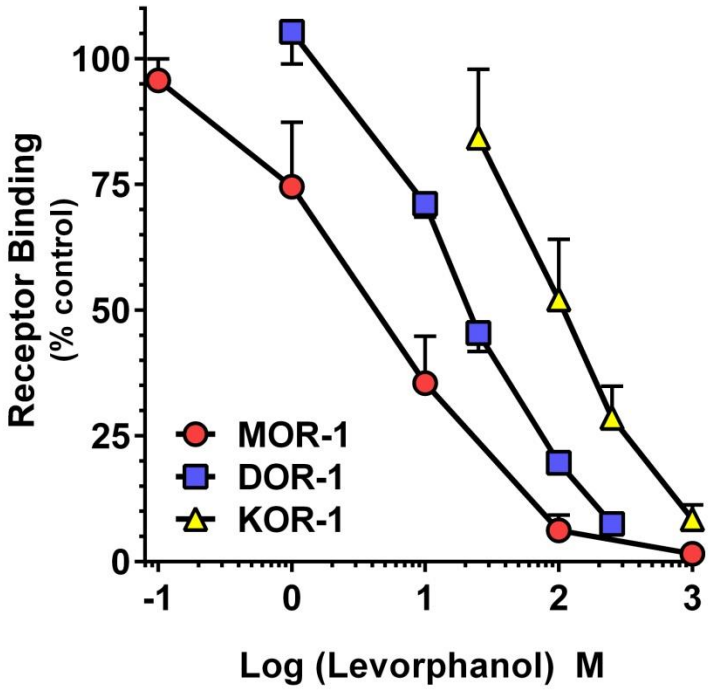


Fig. 2: Competition of mu, delta and kappa receptor binding by levorphanol

Levorphanol competition studies were carried out against stably expressing MOR-1, DOR-1 or KOR-1 CHO cells as described in Methods. Results are pooled from 3 independent replications. Nonlinear regression analysis (Prism) yielded IC_{50} values with 95% confidence limits: MOR-1, 4.4 nM (3.1, 6.3); KOR-1, 108 nM (81, 145); DOR-1, 23.2 nM (19.3, 27.9).

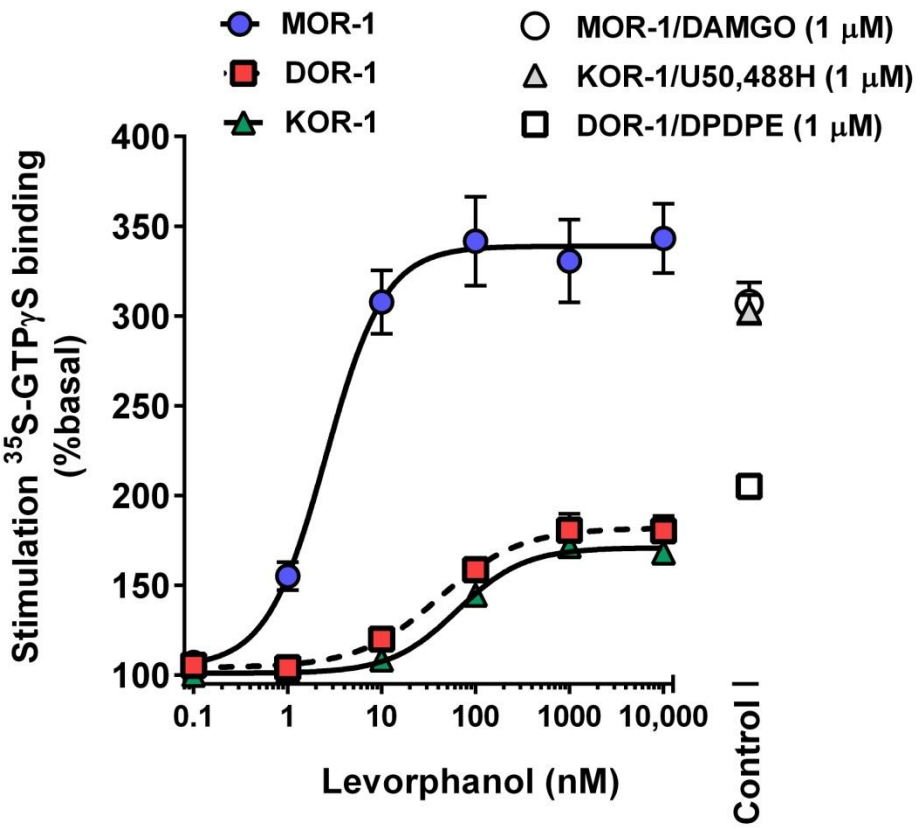


Fig. 3: Levorphanol stimulation of ^{35}S-GTPγS binding

Levorphanol stimulation of ^{35}S-GTPγS binding in CHO cells expressing MOR-1, DOR-1 or KOR-1 receptors was assessed as described in Methods. Results are pooled from three independent experiments and are reported as the percent increase over basal levels. Each assay had an internal control (MOR-1, DAMGO; DOR-1, DPDPE; KOR-1, U50,488H) at 1 μM, which are represented on the figure. Analysis of the curves was carried out using nonlinear regression analysis (Prism) and is presented in Table 2.

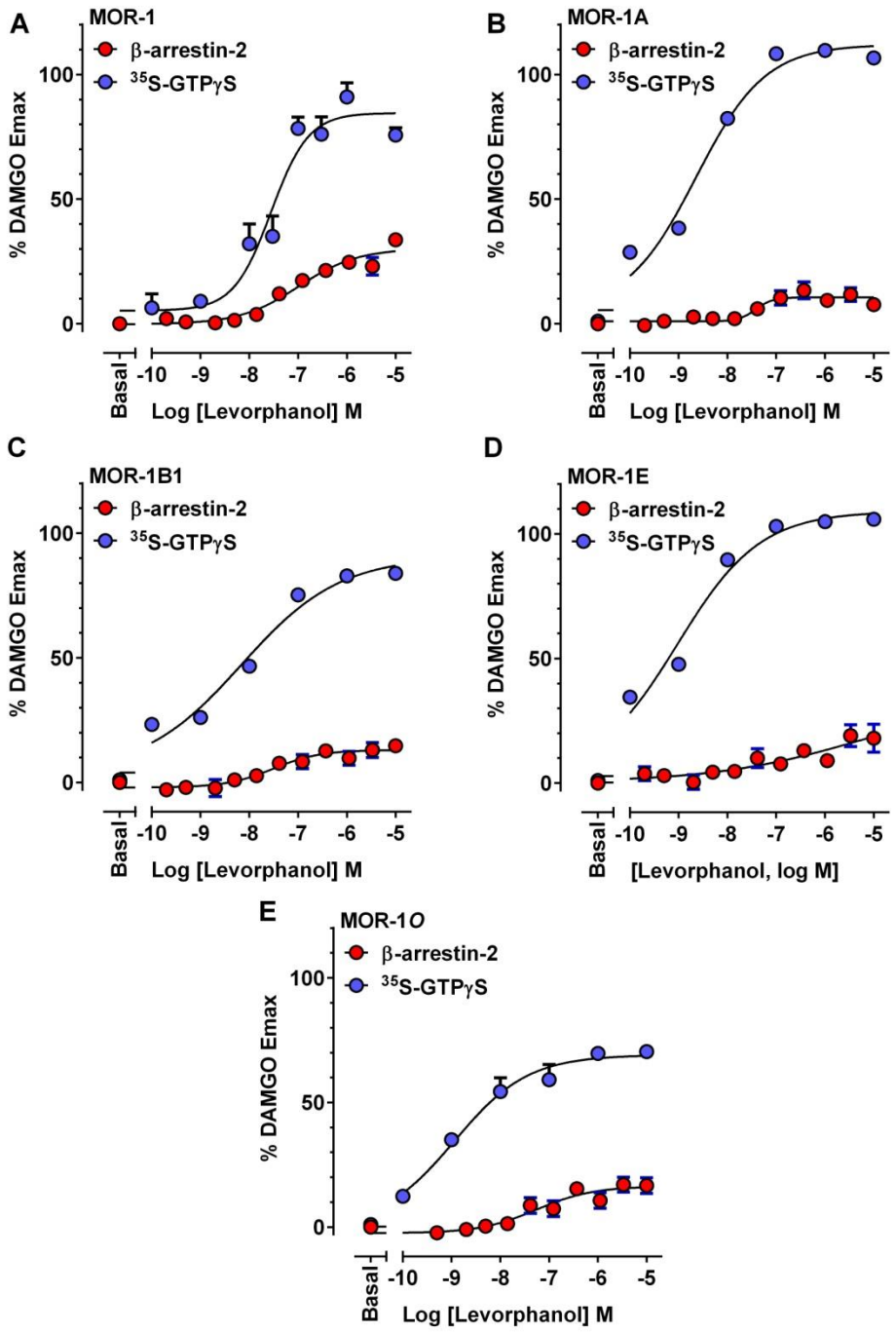


Fig. 4: Levorphanol stimulation of ^{35}S-GTPγS binding and β-arrestin2 recruitment in MOR-1 variants

^{35}S-GTPγS binding and β-arrestin2 recruitment were carried out in transfected CHO cells engineered for the PathHunter assay, as described in Methods and the legend to Table 3. Results are pooled from three independent determinations. Analysis of the data was carried out using Prism and are presented in Table 3.