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ORIGINAL ARTICLE

Tapentadol shows lower intrinsic efficacy at μ receptor than morphine and oxycodone

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Abstract

Tapentadol is a centrally acting analgesic with a dual mechanism of action. It acts as an agonist at the μ receptor and inhibitor of noradrenaline reuptake. Clinical trials suggest similar analgesic efficacy of tapentadol, oxycodone, and morphine in acute and chronic pain. Given the limited information about the molecular actions of tapentadol at the μ receptor, we investigated the intrinsic efficacy of tapentadol and compared it with other opioids. β-chlornaltrexamine (β-CNA, 100 nM, 20 min) was used to deplete spare receptors in AtT20 cells stably transfected with human μ receptor wild-type (WT). Opioid-mediated changes in membrane potential were measured in real-time using a membrane potential-sensitive fluorescent dye. Using Black and Leff's operational model, intrinsic efficacy relative to DAMGO was calculated for each opioid. Tapentadol (0.05 ± 0.01) activated the GIRK channel with lesser intrinsic efficacy than morphine (0.17 ± 0.02) and oxycodone (0.16 ± 0.02). We further assessed the signaling of tapentadol in the common μ receptor variants (N40D and A6V) which are associated with altered receptor signaling. We found no difference in the response of tapentadol between these receptor variants.

KEYWORDS
μ receptor, G protein, intrinsic efficacy, operational model, opioids, pain, receptor depletion, signaling, single nucleotide polymorphism

1 | INTRODUCTION

Opioids have been a vital component of pain relief in acute and severe pain despite the concerns of life-threatening adverse effects. Their use in chronic pain is limited because of the risk of development of tolerance and dependence on long-term use, as well as the potential development of hyperalgesic adaptations.[1] In addition, neuropathic pain is less responsive to opioids. One strategy for developing drugs with similar analgesic action and fewer adverse effects than the prototypical opioid morphine is to synthesize drugs with a dual mechanism of action. An example of that is tramadol which has a combined mechanism of action of μ receptor activation and inhibition of serotonin (5-HT) and norepinephrine reuptake.[2] However, there are two potential drawbacks of tramadol; firstly, the drug is a prodrug and secondly, it is a mixture of two enantiomers which differently contribute to its dual mechanisms of action. (+) Tramadol preferentially causes serotonin

Abbreviations: CHO cells, Chinese Hamster Ovary cells; ERK, extracellular-signal-regulated kinase; GIRK, G protein-gated inwardly rectifying K^+ channel; SNP, single nucleotide polymorphism; SRIF, somatotropin release inhibiting factor (somatostatin); WT, wild-type; β-CAN, β-chlornaltrexamine.

reuptake inhibition whereas (-) tramadol inhibits norepinephrine reuptake.[3] (+) Tramadol is primarily metabolized by CYP2D6 to its active metabolite, (+)-o-desmethyl tramadol which has a higher affinity at the μ receptor than its parent compound.[4] The gene for CYP2D6 is highly polymorphic leading to variable therapeutic responses to tramadol.[5,6] An alternative approach to overcome the shortcomings of tramadol led to the synthesis of tapentadol, which is an active drug having both μ opioid activity and norepinephrine reuptake inhibition in a single chemical molecule.[7]

Tapentadol is prescribed as an alternative to other typical opioid analgesics for chronic and acute pain. The safety, efficacy, and tolerability of tapentadol have been assessed in human pain states. A Cochrane meta-analysis with pain relief as the primary outcome suggested tapentadol is not superior to morphine or oxycodone for cancer pain.[8] However, the relative contribution of each mechanism of action for the observed clinical efficacy of tapentadol is not clear.

The μ receptor is the primary molecular target for most clinically used opioid analgesics. It is a Gαi/o specific G protein-coupled receptor (GPCR) encoded by the OPRM1 gene.[9] Several non-synonymous single nucleotide polymorphisms (SNPs) have been identified in this gene, of which the most common ones are A118G (N40D) and C17T (A6V).[10] These common variants may result in individual differences in the clinical response to opioids. Both the efficacy and potency of buprenorphine were markedly reduced in CHO cells expressing N40D in assays measuring inhibition of cAMP and ERK phosphorylation, whereas signaling of many opioids including morphine, buprenorphine, and fentanyl was negatively impacted in a similar study conducted in CHO cells expressing A6V.[11,12] Many studies have examined the potential consequence of these SNPs for receptor expression and function. The findings from these in vitro studies are inconsistent (reviewed in Knapman et al.),[10] and one of the potentially significant confounders may be the presence of different receptor reserves in different cell systems.

In the present study, we depleted the spare μ receptors in AtT20 cells expressing human μ receptor (μ-WT) using an irreversible antagonist. We measured the intrinsic efficacy of the tapentadol and other agonists relative to DAMGO in an assay of GIRK channel activation. We further assessed the effect of common μ receptor variants in the signaling of tapentadol and other opioids. This is the first study that uses intrinsic efficacy to compare tapentadol with other opioid agonists and to investigate whether tapentadol shows a similar effect at μ receptor variants.

2 | MATERIALS AND METHODS

2.1 | Cell culture

Mouse pituitary tumor cells, AtT20 (ATCC CRL-1795) stably expressing the human μ receptor wild-type (WT), N40D and A6V were previously created using the FlpIn system (Invitrogen) in our lab.[11,12] These cells were grown and maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, and with selection antibodies (80 μg/ml hygromycin). Zeocin 100 μg/ml was used for the maintenance of AtT20 FlpIn WT cells. The cells were maintained and stored at 37°C, 5% CO_2 humidified incubator. They were passaged upon reaching 80% confluency and were used until the 20th passage.

A day before the experiment, the cells were trypsinized, centrifuged, and resuspended in Leibovitz's L-15 media containing 1% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 15 mM glucose. The cell suspension was loaded onto black-walled clear bottomed 96 well plates precoated with 1 μg/ml poly-D-lysine (Sigma–Aldrich) and incubated overnight at 37°C with ambient CO_2 in a humidified incubator.

2.2 | Membrane potential assay

The intrinsic efficacy of the opioid agonists was measured using receptor depletion assay. This involves the use of an irreversible μ receptor antagonist, β-chlornaltrexamine (β-CNA), to permanently deplete the receptor reserve in AtT20 human μ cells. β-CNA binds and alkylates μ receptors, and ultimately inactivates them.[13] We optimized the concentration and incubation time of β-CNA to produce a reduction of the maximum effect of a high efficacy agonist, Tyr-D-Ala-Gly-*N*-MePhe-Gly-ol acetate (DAMGO), producing a condition where no spare receptors are present for the studied drugs.

On the day of the assay, 100 nM β-CNA was prepared in cold modified Hank's Buffered Salt Solution (HBSS) composed of (in mM) NaCl 145, HEPES 22, Na_2HPO_4 4.17, KH_2PO_4 0.441, $MgSO_4$ 0.407, $MgCl_2$ 0.493, $CaCl_2$ 1.26, and glucose 5.56 (pH 7.4 and osmolarity 315 ± 15). L-15 was removed, and cells were treated with 100 nM β-CNA or HBSS (control) for 20 min. After incubation, washes were performed twice using warm HBSS. Warm L-15 and FLIPR® membrane potential assay dye (blue, used at 50% of the manufacturer's recommended concentration, Molecular Devices, Sunnyvale, CA) reconstituted in HBSS (90 μl each) were added to the cells and incubated at 37°C for an hour. Various concentrations of drugs were prepared at 10 times the final concentration in HBSS by serial dilution. Fluorescence changes were measured using FlexStation 3 plate reader (Molecular Devices) with excitation and emission set at 530 nm and 565 nm, respectively. The baseline was measured for 120 s at an interval of 2 s. At 120 s, 20 μl of the drug was added and the response was recorded for a total of 180 s.

Somatostatin (SRIF-14) and ML-297 effects in AtT20 WT cells were also measured using membrane potential assay (without receptor depletion). Tapentadol was added at 120 s followed by SRIF or ML-297 at 420 s. The response was recorded for further 300 s.

2.3 | Data analysis

GIRK channel activation was expressed as a percentage change in fluorescence from baseline after subtraction of changes produced

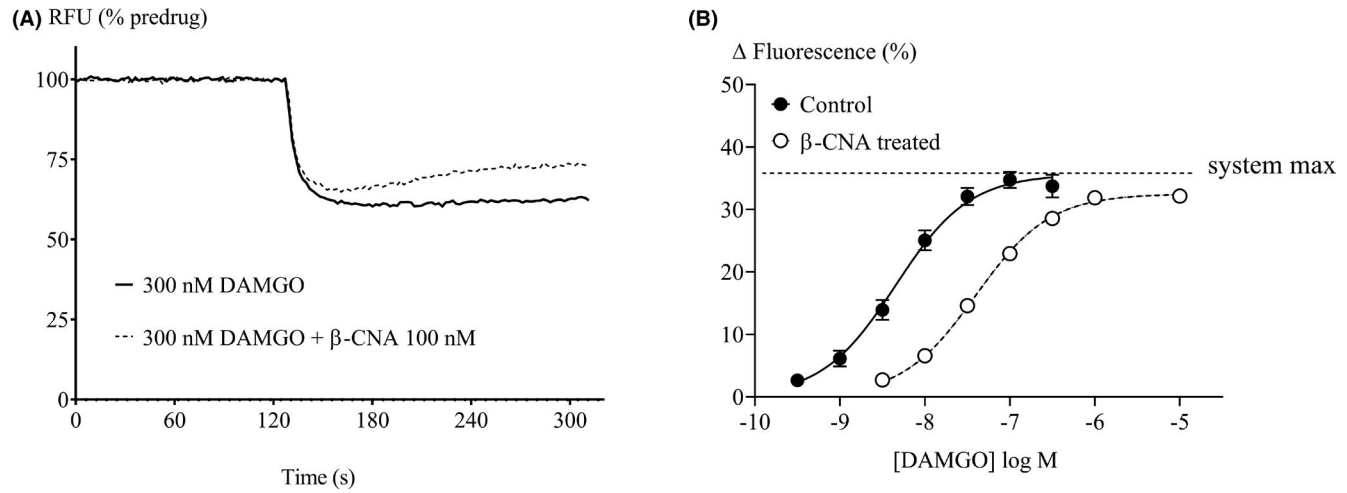

FIGURE 1 The effect of β-CNA in DAMGO induced hyperpolarisation in AtT20 μ-WT cells. Cells were pre-treated with β-CNA (100 nM), or HBSS for 20 min. (A) Representative traces normalised to baseline reading showing a decrease in fluorescence upon treatment with DAMGO with (dashed line) and without β-CNA (solid line). (B) Pre-treatment of cells with β-CNA causes rightward displacement of CRC of DAMGO. Data represent the mean ± SEM of five independent experiments, each in duplicate

by the HBSS alone. The values were normalized to the maximum individual response of DAMGO without receptor depletion. The concentration-response curve (CRC) before and after depletion was obtained from GraphPad Prism Version 8, and the curves simultaneously fitted to Black and Leff's operational models of pharmacological agonism[14] with Hill slope constrained to one and bottom constrained to zero. *Tau* relative to DAMGO was calculated as the ratio of *tau* of agonist to the *tau* of DAMGO.

The equation of the operational model of depletion used to obtain the *tau* (transducer constant) and K_A (agonist-receptor dissociation constant) as presented in GraphPad prism is as follows:

$$Y = \frac{\text{Basal} + (\text{Effect}_{max} - \text{Basal})}{1 + \text{operate}}, \text{where,}$$

$$\text{operate} = \left(\frac{KA + X}{\tau + X}\right)^n$$

Here, *tau* represents the inverse of the fraction of receptors needed to be occupied by the agonist to show half its maximal response, and K_A represents functional affinity. The parameter of *tau* is composed of tissue and non-tissue (or drug) components. To cancel out the tissue-dependent factors which may account for variability in in vitro experiments using different cell lines, we calculated relative *tau* for each agonist taking DAMGO as the standard. The relative *tau* for an agonist at an equivalent fraction of receptors in a cell line gives a measure of relative intrinsic efficacy (as reviewed by Kelly, 2013).[15]

For double addition assay using ML-297 and SRIF in AtT20 WT cells, data were analyzed as percentage change produced by SRIF or ML-297 alone after blank subtraction. Data for all the experiments were expressed as mean ± SEM of at least six independent experiments conducted in duplicates unless otherwise stated. Shapiro-Wilk test was performed to confirm normal distribution for all the data sets. Statistical analysis was executed using one-way ANOVA and Dunnett's multiple comparisons post hoc test where $p < .05$ was considered significant.

2.4 | Drugs and chemicals

DAMGO and somatostatin were purchased from Auspep. Morphine, oliceridine (TRV-130), and β-CNA were purchased from GlaxoSmithKline, AdooQ Bioscience, and Sigma–Aldrich, respectively. Buprenorphine and oxycodone were from the National Measurement Institute and O-desmethyl tramadol from Toronto Research Chemicals Inc. (TRC). Tapentadol was supplied by Cayman Chemicals. The selection antibiotics are from Invivogen. All the tissue culture reagents were from Thermo Fisher Scientific unless otherwise stated.

2.5 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[16] and are permanently archived in the Concise Guide to PHARMACOLOGY (2019/20).[17]

3 | RESULTS

3.1 | Effect of receptor depletion in DAMGO induced hyperpolarization

β-CNA is an irreversible antagonist of opioid receptors.[18,19] The membrane potential assay was performed for DAMGO after treating the cells with 100 nM β-CNA as described previously.[20] Inactivating a fraction of the receptors caused a decrease in DAMGO-induced hyperpolarization (Figure 1A). Data were analyzed using Black and Leff's operational model and CRC was plotted (Figure 1B). Pre-treatment with β-CNA resulted in a significant rightward

displacement of DAMGO CRC. The *tau* value of DAMGO ($n = 19$) for control and β-CNA treated was 198.0 ± 38.0 and 19.7 ± 5.4, respectively, which means at the depleted system, 10 times more receptors need to be occupied by DAMGO to elicit half its maximal response.

3.2 | Comparing the relative intrinsic efficacy of tapentadol with other opioids in AtT20 μ-WT cells

This experimental design was used to investigate the operational efficacy of tapentadol, morphine, O-desmethyl tramadol, oxycodone, oliceridine (TRV-130), and buprenorphine in AtT20 μ-WT cells. All the opioids produced a hyperpolarisation of AtT20 cells in a concentration-dependent manner, and there was a significant rightward displacement of the concentration-response curves after β-CNA treatment (Figure 2). Using the operational model of depletion, we calculated the corresponding *tau* value for each opioid. The relative *tau* and K_A values of the opioids are listed in Table 1.

Tapentadol produced a hyperpolarization with significantly less intrinsic efficacy than morphine and oxycodone in cells expressing μ-WT. Its effect was not different from the low efficacy μ-agonists buprenorphine and oliceridine. Buprenorphine, morphine, and oliceridine had K_A values less than 50 nM whereas tapentadol demonstrated a low micromolar affinity (5 μM) for the WT receptor (Table 1).

3.3 | Effect of SNPs in the response of tapentadol and conventional opioids

We further assessed the response of opioids in AtT20 cells expressing μ receptor variants N40D and A6V. β-CNA was able to knock down the maximum effect of the high efficacy agonist, DAMGO in both cell lines (Figure S1), and the effect of DAMGO was not different in variants as compared to WT.

The effect of all opioids to activate the GIRK channel was not different across cell lines. Tapentadol produced hyperpolarization with significantly lower intrinsic efficacy than morphine and oxycodone in cells expressing N40D and A6V (Figures 3 and 4, and Table 1). The intrinsic efficacies of all the opioids under study have been compiled in the scattered dot plot (Figure 5). The opioids formed two clusters based on intrinsic efficacy which is consistent among all three cell lines.

To compare the concentration-response curves in more detail, we compiled the EC_{50} and E_{max} for all opioids across all cell lines in Table S1.

3.4 | Higher concentration of tapentadol alters the GIRK activation signal

In undepleted conditions, the concentration-response curve for tapentadol reached a plateau at concentrations of 3 and 10 μM, but the highest concentration of tapentadol (30 μM) tested produced a smaller response, similar to 1 μM (Figure 6). We hypothesized that 30 μM is either directly interfering with the GIRK channel or with the signal from receptor to channel. Hence, we investigated the effect of 30 μM tapentadol on responses to somatostatin (SRIF, 100 nM) and ML297 (30 μM) in AtT20 WT cells. Somatostatin receptors are endogenously expressed in AtT20 cells and can open GIRK channels similar to μ receptors while ML297 is a direct and selective GIRK1/2 channel activator. It is noted that 30 μM tapentadol caused a significant reduction in the response of both SRIF (Figure 7A,B $p = .016$, unpaired Student's *t*-test) and ML297 (Figure 7C,D $p = .015$, unpaired Student's *t*-test).

4 | DISCUSSION

The present study compares the relative efficacy of tapentadol with conventional opioids to activate the GIRK channel in AtT20 μ-WT cells. We concluded that tapentadol is a low efficacy μ agonist at all three μ receptor variants tested (WT, N40D, and A6V), and it has lower efficacy than oxycodone and morphine. To our knowledge, this study provides the first quantification of relative intrinsic efficacy of tapentadol at the μ receptor.

The efficacy of a ligand depends on the action of a drug upon binding to the receptor and tissue factors such as transducers and receptor expression.[15] The expression of a large number of receptors in tissue may result in lower efficacy agonists binding to spare receptors and eliciting a maximum response of the system. In the present study, we depleted the spare receptors using an irreversible antagonist and used the operational model of agonism to obtain *tau* value, which is the ratio of the number of receptors in tissue to the number of receptors occupied by an agonist to elicit half its maximal response.[21] *Tau* does not exclude system-dependent variables hence, estimating relative *tau* using DAMGO becomes a consistent measure of the operational efficacy of a drug irrespective of the tissue being analyzed or assay used.[15]

The value of *tau* for some opioids has been previously calculated with GTPγS binding assay in HEK293 cells expressing rat μ receptor using the operational model of agonism.[22] The operational *tau* for DAMGO, morphine, oxycodone, and buprenorphine in that study was 28.5 ± 1.1, 5.2 ± 0.2, 5.1 ± 0.2, and 0.6 ± 0.1, respectively. We calculated the *tau* of the compounds relative to DAMGO from their result and found it to be 0.18 for morphine, 0.17 for oxycodone, and 0.02 for buprenorphine, which is consistent with our findings (Table 2). We included the data from this particular study as it tested four of the opioids we used in our assay. Few other studies have used the operational model to calculate the intrinsic efficacy, but they only have morphine in common with our study. Moreover, the average value of intrinsic efficacies of morphine relative to DAMGO calculated using the operational model by five different studies was shown to be 0.19 ± 0.08.[15] The similar efficacy in different assays in different tissues highlights the importance of relative τ as a standard measurement of efficacy.

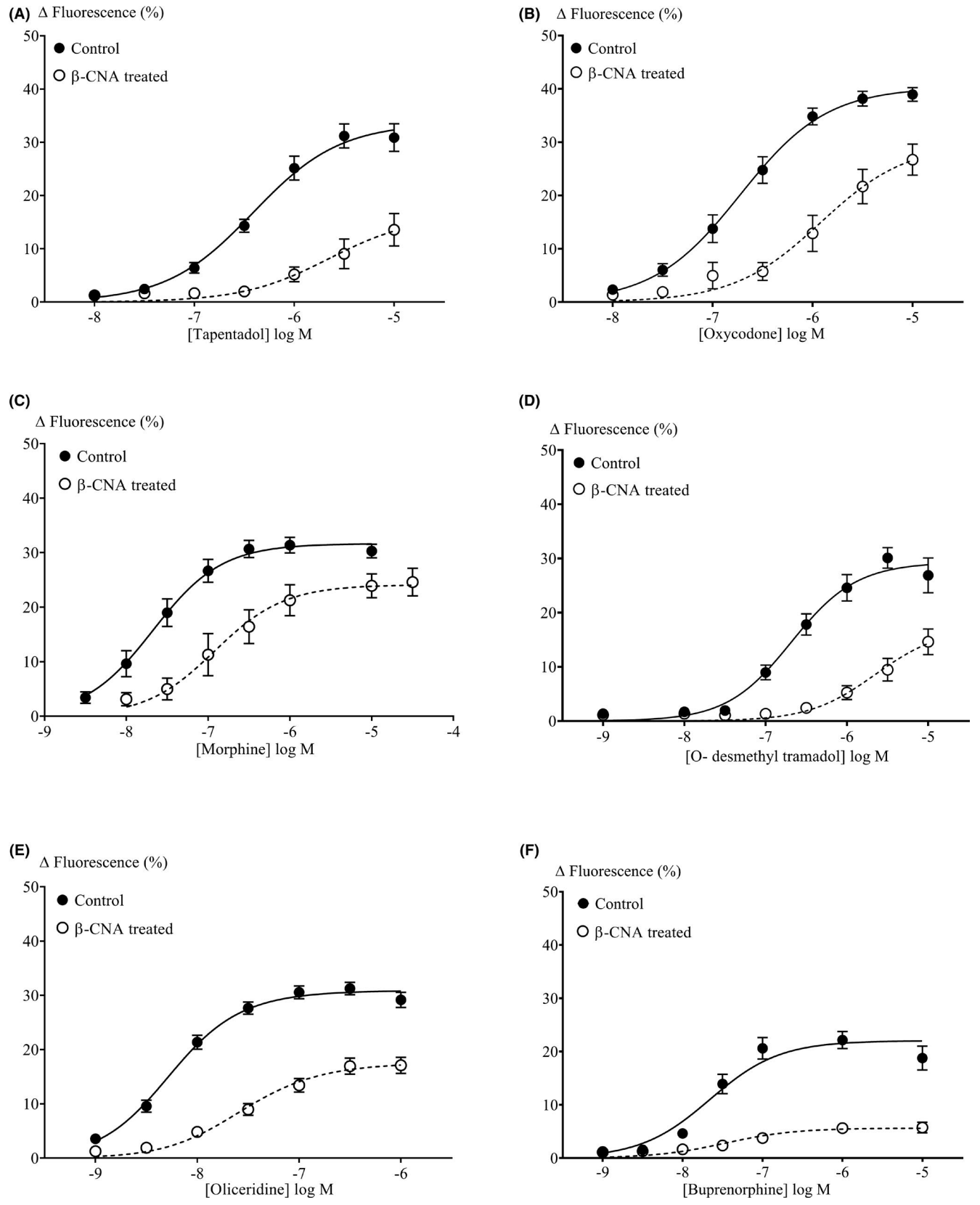

FIGURE 2 Activation of GIRK channel by opioids in AtT20 μ-WT cells. Concentration-response curves for (A) tapentadol, (B) oxycodone, (C) morphine, (D) o- desmethyl tramadol, (E) oliceridine and (F) buprenorphine were plotted for control (solid line) and β-CNA (dashed line) treated response. Data represent the mean ± SEM of six independent experiments, each in duplicate

TABLE 1 Summary of the intrinsic efficacy and operational affinity values for opioids tested

	WT		N40D		A6V	
Opioids	**Relative *tau***	**pKA**	**Relative *tau***	**pKA**	**Relative *tau***	**pKA**
Tapentadol	0.05 ± 0.01	5.4 ± 0.3	0.05 ± 0.02	5.4 ± 0.3	0.04 ± 0.01	5.35 ± 0.3
O-Tramadol	0.08 ± 0.02	5.33 ± 0.4	0.03 ± 0.00	5.38 ± 0.34	0.08 ± 0.04	5.55 ± 0.3
Oliceridine	0.09 ± 0.03	7.45 ± 0.2	0.06 ± 0.01	7.44 ± 0.13	0.05 ± 0.01	7.47 ± 0.18
Buprenorphine	0.02 ± 0.008	7.42 ± 0.4	0.008 ± 0.001	7.49 ± 0.54	0.02 ± 0.008	7.69 ± 0.44
Morphine	0.18 ± 0.02 $p = .002$	6.38 ± 0.3	0.16 ± 0.03 $p = .047$	6.27 ± 0.18	0.21 ± 0.06 $p = .02$	6.24 ± 0.18
Oxycodone	0.16 ± 0.03 $p = .006$	5.39 ± 0.3	0.16 ± 0.04 $p = .009$	5.44 ± 0.26	0.24 ± 0.06 $p = .004$	5.39 ± 0.26

Note: The values of relative *tau* that were significantly different from tapentadol as analyzed after multiple comparisons using one-way ANOVA are highlighted in red.

Electrophysiological recordings of GIRK currents from rat locus coeruleus (LC) neurons were used to demonstrate that tapentadol has a lower intrinsic activity than morphine and oxycodone at rat μ receptor, which is consistent with our conclusion in human μ receptor.[23] LC neurons are useful to study the pharmacology of tapentadol because of the co-expression of μ receptors and noradrenaline transporters. It is noteworthy that this study[23] compared relative intrinsic activity, which is different from the intrinsic efficacy investigated in the present study. The intrinsic activity provides a reasonable estimation of efficacy in tissue with no spare receptors.[15] Although the presence of receptor reserve in LC neurons is well documented,[24] it does not interfere with morphine, tapentadol, and oxycodone efficacy measurement as they have submaximal responses (no spare receptors).

The in vitro efficacy of tapentadol at human μ receptor has also been measured using the [^{35}S] guanosine 5′-3-O-(thio)triphosphate (GTPγS) binding assay. This study reported that tapentadol has the same efficacy as morphine, although absolute changes in GTPγS binding were not reported, and the drugs were not compared to a higher efficacy agonist.[7] GTPγS is a robust functional assay, but its sensitivity is strongly regulated by assay conditions, especially the concentration of GDP, sodium, and magnesium ions.[25] The added GDP decreases the basal signal by occupying the empty nucleotide-binding site.[26] Thus, a low concentration of GDP (1 μM) used in the study may increase the signal from a lower efficacy agonist such as tapentadol because of less competition for [^{35}S] GTPγS. It is also possible that the expression levels of μ receptors in these cells were high enough to saturate the available G proteins, effectively creating a system with spare receptors.[27]

The present study established the presence of two clusters of clinically used opioids based on intrinsic efficacy. The lower efficacy agonists such as buprenorphine, tramadol, tapentadol, and oliceridine formed one cluster distinct from the second cluster formed by the higher efficacy agonist, morphine, and oxycodone. Gillis et al.[20] showed a positive correlation between low intrinsic efficacy and improved side effect profile of opioids through various in vitro and animal studies. Over the clinical concentration range, buprenorphine, tramadol, and oliceridine are reported to show improved tolerability and reduced overdose liability as compared to morphine or oxycodone.[28–30] The reported cases of favorable safety and tolerability in terms of low tapentadol abuse,[31–33] could in part be due to its lower intrinsic efficacy similar to buprenorphine, oliceridine, and tramadol. At the cellular level, opioid-decreased tolerability has been associated with increased β-arrestin recruitment.[34] There is no information in the literature on the effect of tapentadol at β-arrestin but considering low intrinsic efficacy at G protein pathway and favorable tolerability profile, tapentadol is likely to show weak β-arrestin recruitment.

Tapentadol is classified as an atypical opioid considering its dual mechanism of action.[35] Tapentadol-induced analgesia is the result of the synergistic contribution of μ receptor agonism and noradrenaline reuptake inhibition.[36,37] The presence of both components within the same compound makes it practically impossible to determine the individual contributions of the opioid and non-opioid components in clinical settings. A study conducted in LC neurons showed similar potency of tapentadol to activate μ receptor (EC50 1.8 μM) and inhibit noradrenaline transporter (EC50 2.3 μM).[23] In an animal study conducted in OPRM1 knock-out mice, the analgesic effect of morphine was completely abolished, whereas tapentadol provided reduced yet significant analgesia in the dose that was highly effective in the wild-type mouse.[38] The present study measured the efficacy of tapentadol at μ receptor and does not consider its effect on noradrenaline transporter. Recently, a study estimated the μ-load of tapentadol considering different in vitro and in vivo approaches and found it to be approximately 40%.[39] μ-load is the μ receptor-mediated effect of a drug as compared to the prototypical opioids like morphine which has a single mechanism of action of μ receptor activation. Despite the lower μ-load and less intrinsic efficacy than morphine and oxycodone, tapentadol has been suggested to be a better choice in clinical cases of neuropathic pain or pain of mixed origin because of its dual mechanism of action.[40–42] This explains the importance of considering analgesic equivalence rather than opioid equivalence in opioid rotation practice.[43]

The individual variation in the response of opioids due to genetic differences has been well documented with more focus on N40D and A6V.[10,44] These amino acid variations located in the N-terminal

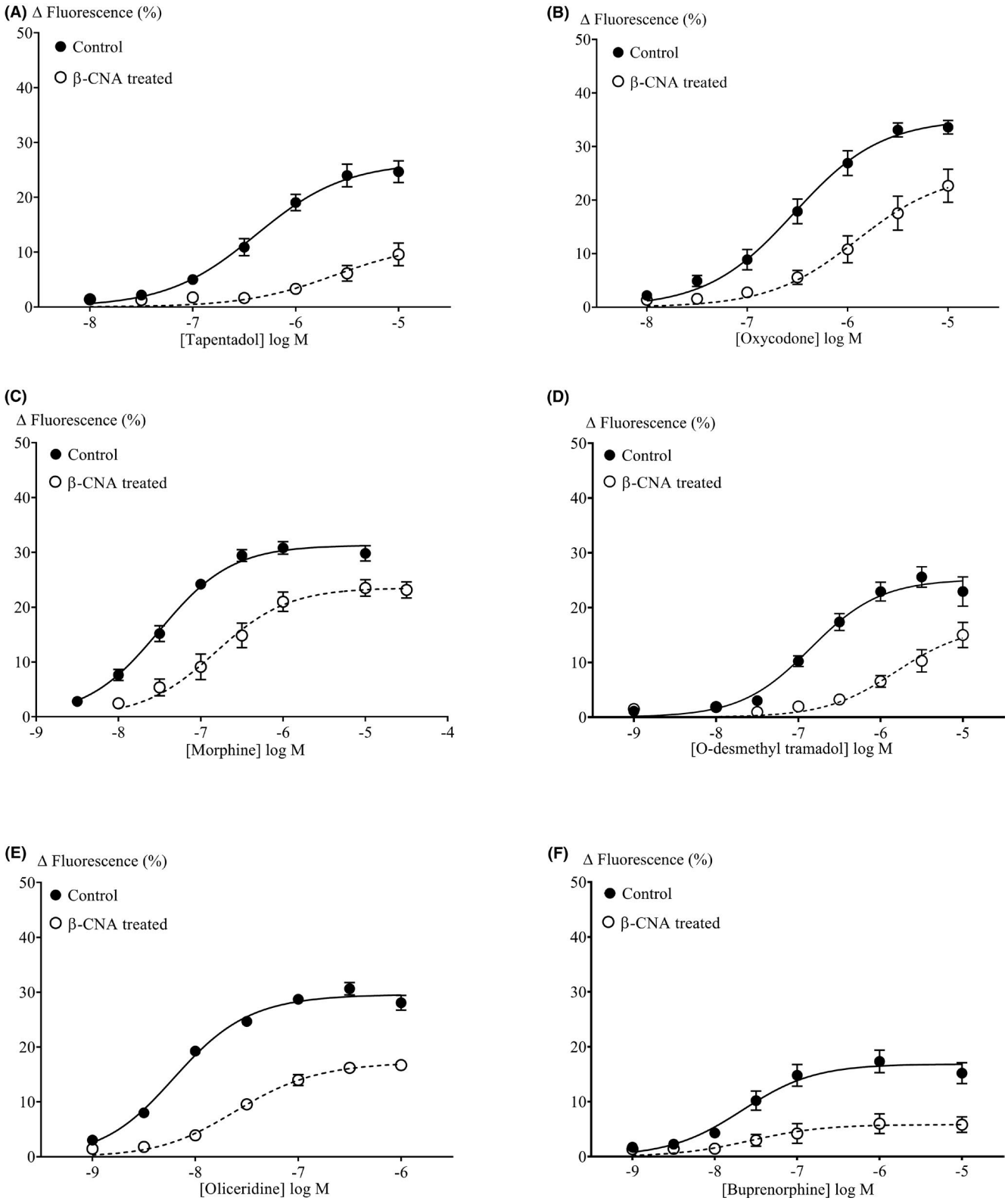

FIGURE 3 Activation of GIRK channel by opioids in AtT20 μ-N40D cells. Concentration-response curves for (A) tapentadol, (B) oxycodone, (C) morphine, (D) O-desmethyl tramadol, (E) oliceridine and (F) buprenorphine were plotted for control (solid line) and β-CNA (dashed line) treated response. Data represent the mean ± SEM of six independent experiments, each in duplicate, conducted in AtT20 μ-N40D cells

domain of the receptor have been linked with altered opioid response in various experimental and clinical settings.[11,12,45] However, many other studies show contrasting results stating no difference between the variants.[46,47] Earlier studies from our lab have shown buprenorphine was most affected in both the SNPs, although the response was pathway and assay-specific.[11,12] In the present study,

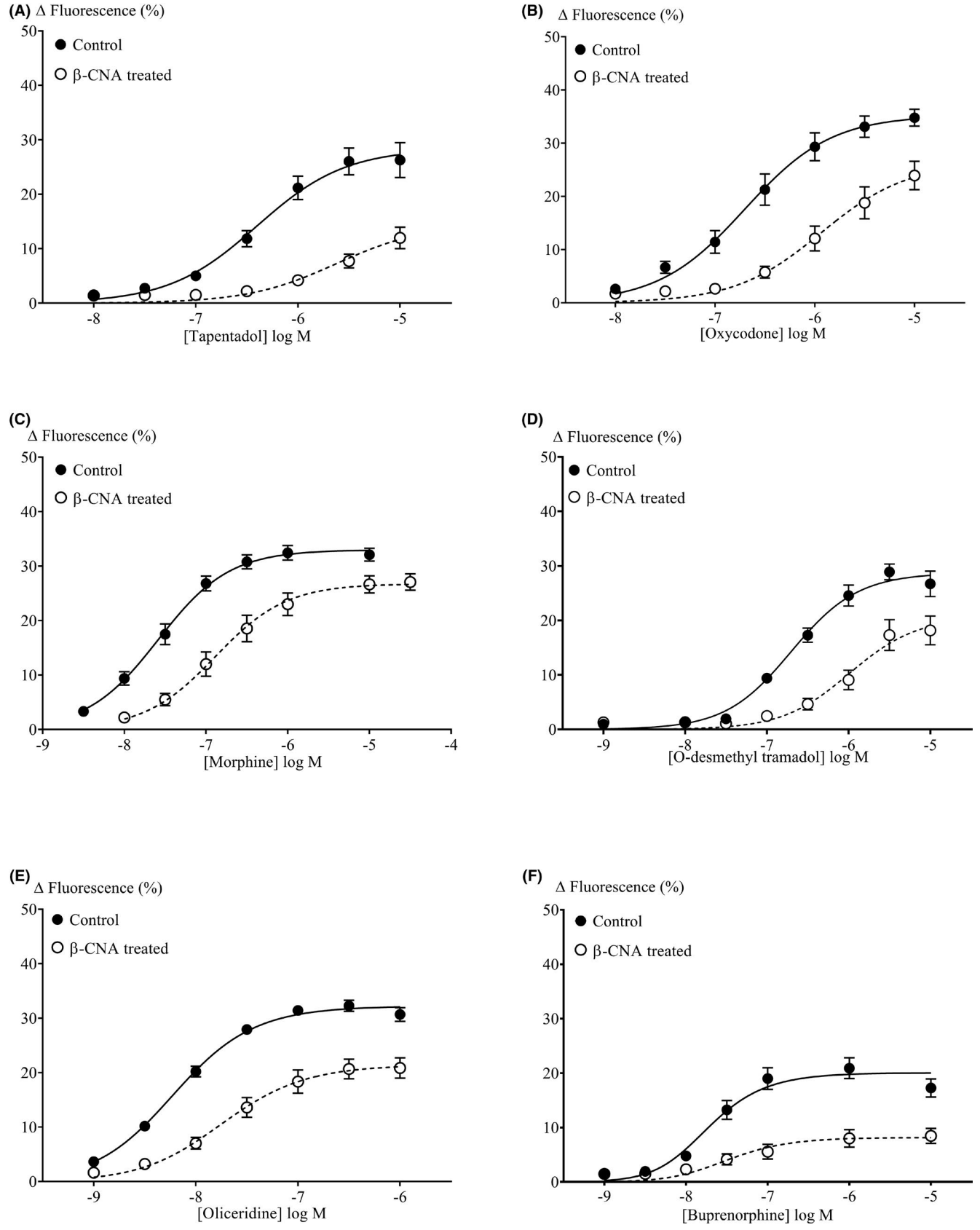

FIGURE 4 Activation of GIRK channel by opioids in AtT20 μ- A6V cells. Concentration-response curves for (A) tapentadol, (B) oxycodone, (C) morphine, (D) O- desmethyl tramadol, (E) oliceridine and (F) buprenorphine were plotted for control (solid line) and β-CNA (dashed line) treated response. Data represent the mean ± SEM of six independent experiments, each in duplicate, conducted in AtT20 μ- A6V cells

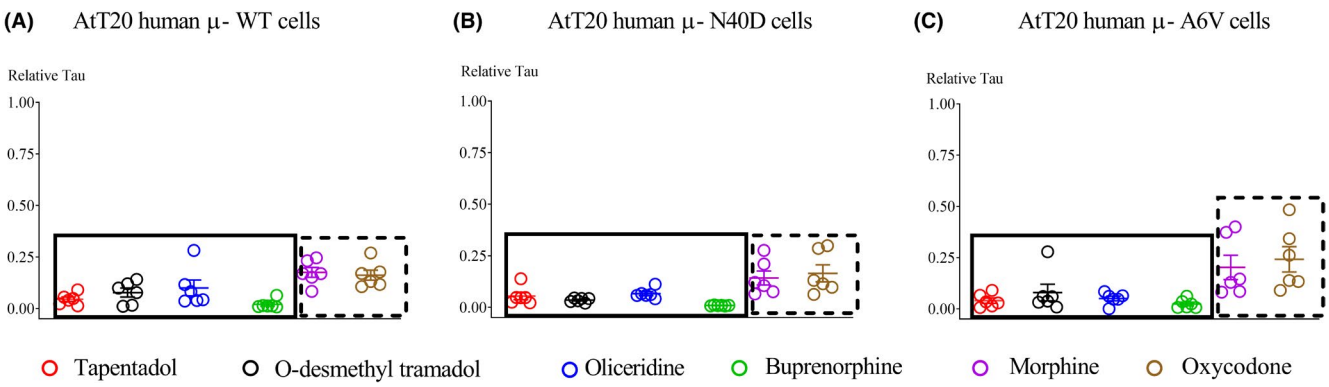

FIGURE 5 Comparison of relative intrinsic efficacy of clinically used opioids in different cell lines. Scatter dot plot showing relative efficacy of tapentadol, tramadol, oliceridine, buprenorphine, morphine, and oxycodone in AtT20 cells stably transfected with human μ receptor (A) WT, (B) N40D and (C) A6V. There is a presence of two distinct clusters of opioids based on relative intrinsic efficacy. The closed box comprising of low intrinsic efficacy opioids such as tapentadol, tramadol, oliceridine and buprenorphine. The second cluster is represented by a dotted box which is comprised of morphine and oxycodone, clinically used opioids with high intrinsic efficacy. The data represents the intrinsic efficacy of opioids relative to DAMGO which has much higher efficacy than all opioids tested

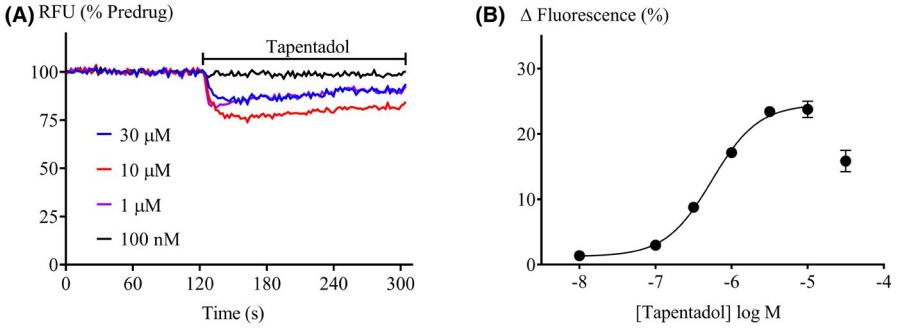

FIGURE 6 Higher concentration of tapentadol interferes with GIRK signal. (A) Representative traces of tapentadol in membrane potential assay. The tested concentrations of tapentadol displayed concentration dependent decrease in fluorescence corresponding to hyperpolarisation except for 30 μM which showed a response similar to 100 nM tapentadol. (B) Concentration response curve of tapentadol without fitting 30 μM in the curve. Data represent mean ± SEM of six independent experiments, each in duplicate

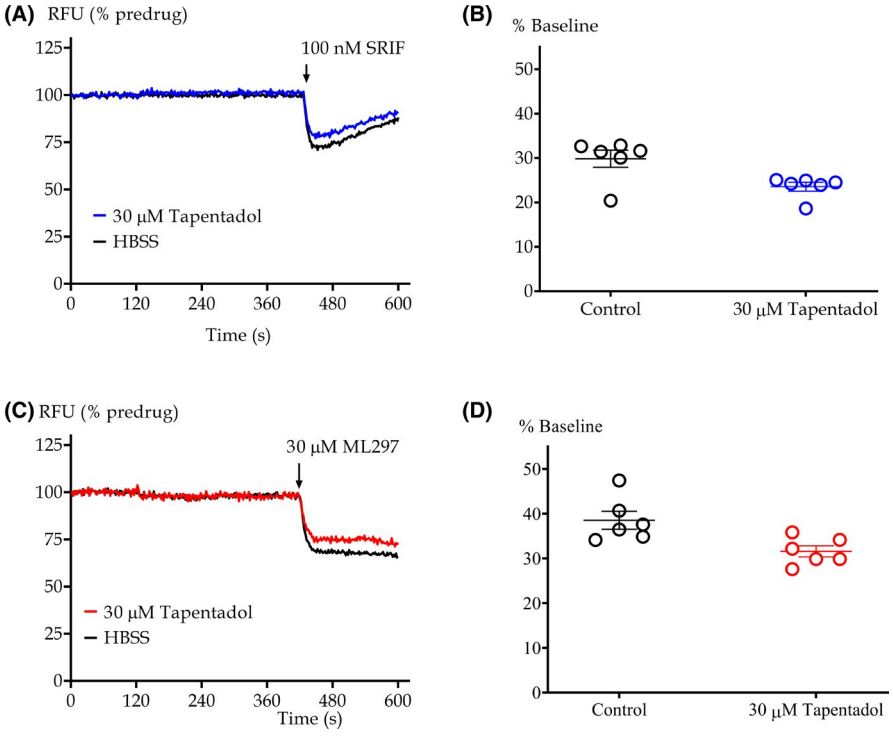

FIGURE 7 Tapentadol at higher concentration decreases SRIF and ML297 response. Raw traces showing the change in fluorescence for (A) 100 nM SRIF and (C) 30 μM ML297 after 5 min incubation with 30 μM tapentadol on AtT20 WT cells. (B) Scatter dot plot showing the percentage change in fluorescence of (B) SRIF and (D) ML297 in the presence of tapentadol. Data represent mean ± SEM of six independent experiments, each in duplicate

TABLE 2 Comparison of relative intrinsic efficacy of selective opioids measured by membrane potential assay and GTPγS binding assay

	Membrane potential assay		GTPγS binding assay[21]	
	Operational *tau*	Relative intrinsic efficacy	Operational *tau*	Relative intrinsic efficacy
DAMGO	90 ± 22		28.5 ± 1.1	
Morphine	15.4 ± 4	0.18 ± 0.02	5.2 ± 0.2	0.18
Oxycodone	13.9 ± 2	0.16 ± 0.02	5.1 ± 0.2	0.17
Buprenorphine	1.9 ± 0.3	0.02 ± 0.008	0.6 ± 0.1	0.02

we used an irreversible antagonist and operational model which eradicates the confounding differences in the system and we found that neither buprenorphine nor any other opioids tested showed a variable response in the cell lines expressing the μ receptor polymorphisms. However, the signaling pathway we examined had previously shown no differences among variants when receptor reserve was not considered,[11,12] and it remains to be determined whether other signaling pathways remain more sensitive to μ receptor polymorphisms.

We identified that at 30 μM concentration, tapentadol causes a decrease in GIRK signaling. In AtT20 cells not expressing μ receptors, 30 μM tapentadol decreased the maximal response of SRIF and ML297. This suggests interference with the activation of GIRK channels, although the exact mechanism is not known. This is in contrast to a study using electrophysiologic recordings in rat LC neurons, where no blockage of GIRK channels was reported at a concentration of 100 μM.[23] Possible reasons for these differences include species differences or a voltage-dependent effect of tapentadol. A voltage-dependent effect of tapentadol may not have been detected in the electrophysiological recording as cells were clamped at −60 mV throughout the experiments, whereas in the membrane potential assay, membrane voltage is free to vary. However, the off-target effect of 30 μM is unlikely to be significant at therapeutic doses, as previous studies have shown the plasma concentration of tapentadol to range between 100 nM and 560 nM.[48,49] However, in rare cases of tapentadol-related fatality, the serum concentration was reported to be 3.5–25 μM.[49–52]In conclusion, this study demonstrates tapentadol to be a lower intrinsic efficacy μ agonist at one of the classical G protein-dependent signaling pathways for the μ receptor. Future studies should be directed at studying the effect of tapentadol on G protein independent pathways such as β-arrestin recruitment.

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DISCLOSURES

The authors declare that they have no conflict of interest related to this work.

AUTHORS CONTRIBUTIONS

PM designed and performed experiments, analyzed the data, and wrote the manuscript. MS and MC provided critical feedback and helped shape the research, analysis, and manuscript. All authors reviewed and edited the manuscript.

ETHICS STATEMENT

This study is exempt from ethics approval.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

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