Toward Directing Opioid Receptor Signaling to Refine Opioid Therapeutics

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Supplemental Information

TABLE S1. Opioid Effects in Genetic Models with Disrupted MOR Regulation

Antinociception and Tolerance

Genetic Model	Drug	Response	Effect Relative to WT	Ref
βarrestin2-KO mice	Morphine	Hot plate latency	Enhanced, prolonged	8,11,10, 23
	Morphine (sc daily, pellet)	Hot plate tolerance	Decreased	9,11
	Morphine (pump)	Hot plate tolerance	Decreased	23
	Morphine	Tail flick latency	Enhanced, prolonged	11,12
	Morphine (sc daily)	Tail flick tolerance	Delayed, but present	11
	Heroin	Hot plate latency	Enhanced, prolonged	10
	Etorphine	Hot plate latency	No change	10
	Fentanyl	Hot plate latency	No change	10, 23
	Fentanyl (pump)	Hot plate tolerance	No change	23
	Oxycodone	Hot plate latency	No change	23
	Oxycodone (pump)	Hot plate tolerance	No change	23
	Methadone	Hot plate latency	No change	23
	Methadone (pump)	Hot plate tolerance	No change	23
βarrestin2 Antigene mice	Morphine	Hot plate latency	Enhanced	13
	Morphine (sc daily)	Hot plate tolerance	Decreased	13
βarrestin2 siRNA PAG mice	Morphine	Hot plate latency	Enhanced	14
	Morphine (sc daily)	Hot plate tolerance	Decreased	14
βarrestin2 siRNA it rats	Morphine	Tail flick latency	Enhanced, prolonged	15
	Morphine (it daily)	Tail flick tolerance	Decreased	15
βarrestin1-KO mice	Morphine	Hot plate latency	No change	10
βarrestin1 siRNA PAG mice	Morphine	Hot plate latency	No change	14
	Morphine (sc daily)	Hot plate tolerance	No change	14
GRK3, 4, or 6-KO mice	Morphine	Hot plate latency	No change	18, 19, 24
GRK3-KO mice	Morphine (pellet)	Hot plate tolerance	No change	24
	Morphine (sc daily)	Hot plate tolerance	No change	19
	Fentanyl	Hot plate latency	No change	24
	Fentanyl (pump)	Hot plate tolerance	Decreased	24
	Fentanyl (sc daily)	Hot plate acute tolerance	Decreased	19
	Etonitazene (sc daily)	Hot plate tolerance	Decreased	19
GRK5-KO mice	Morphine	Hot plate latency	Decreased	19
	Morphine (sc daily)	Hot plate tolerance	No change	19
GRK6-KO mice	Morphine (pump)	Hot plate tolerance	No change	23
Phosphorylation deficient C terminus of MOR mice	Morphine	Hot plate latency	Enhanced	21
	Morphine (pump)	Hot plate tolerance	Decreased	21
	Fentanyl	Hot plate latency	Enhanced	21
	Fentanyl (pump)	Hot plate tolerance	Decreased	21
MOR S375A mice	Fentanyl (sc daily)	Hot plate tolerance	Decreased	25
	Etonitazine (sc daily)	Hot plate tolerance	Decreased	25
	Morphine (sc daily)	Hot plate tolerance	No change	25
MOR C-terminus truncation mice	Morphine (sc daily)	Tail flick tolerance	Decreased	22

Physical Dependence and Withdrawal

βarrestin2-KO mice	Morphine (pellet)	Naloxone-precipitated withdrawal	No change	9
	Morphine (pump)	Naloxone-precipitated withdrawal	Decreased	23
βarrestin2 siRNA i.t. rats	Morphine (it daily)	Naloxone-precipitated withdrawal	Decreased	15
GRK3-KO mice	Fentanyl (pump)	Naloxone-precipitated withdrawal	No change	24
GRK5-KO mice	Morphine (sc daily)	Naloxone-precipitated withdrawal	Decreased	19
GRK6-KO mice	Morphine (pump)	Naloxone-precipitated withdrawal	No change	20
S375A mice	Morphine (pump)	Naloxone-precipitated withdrawal	No change	21
	Fentanyl (pump)	Naloxone-precipitated withdrawal	No change	21
Phosphorylation deficient C terminus of MOR mice	Morphine (pump)	Naloxone-precipitated withdrawal	No change	21
	Fentanyl (pump)	Naloxone-precipitated withdrawal	No change	21
MOR C-terminus truncation mice	Morphine (sc daily)	Naloxone-precipitated withdrawal	Decreased	22
exon4-encoded C terminus deletion mice	Morphine (sc daily)	Naloxone-precipitated withdrawal	Decreased	22
exon7-encoded C terminus deletion mice	Morphine (sc daily)	Naloxone-precipitated withdrawal	No change	22

Constipation

βarrestin2-KO mice	Morphine	Accumulation of fecal boli	Increased	33
		Small intestinal transit - orally administered charcoal meal	No change	33
		Large intestine transit - colonic bead expulsion	Decreased delay	33
GRK6-KO mice	Morphine	Accumulation of fecal boli	Increased	20
		Small intestinal transit - orally administered charcoal meal	No change	20
		Large intestine transit - colonic bead expulsion	Decreased delay	20
MOR S375A mice	Morphine	Accumulation of fecal boli	No change	21
	Fentanyl	Accumulation of fecal boli	No change	21
Phosphorylation deficient C terminus of MOR mice	Morphine	Accumulation of fecal boli	No change with enhanced potency in constipation	21
	Fentanyl	Accumulation of fecal boli	No change	21
MOR C-terminus truncation mice	Morphine	Small intestinal transit - orally administered charcoal meal	No change	22
exon4-encoded C terminus deletion mice	Morphine	Small intestinal transit - orally administered charcoal meal	Increased distance	22
exon7-encoded C terminus deletion mice	Morphine	Small intestinal transit - orally administered charcoal meal	No change	22

Respiratory Suppression

βarrestin2-KO mice	Morphine	Respiratory suppression	Decreased	33
MOR S375A mice	Morphine	Respiratory suppression	No change	21
	Fentanyl	Respiratory suppression	No change	21
Phosphorylation deficient C terminus of MOR mice	Morphine	Respiratory suppression	No change with enhanced potency in respiratory suppression	21
	Fentanyl	Respiratory suppression	No change with enhanced potency in respiratory suppression	21

Running Behaviors and Reward

βarrestin2-KO mice	Morphine	Locomotor activity	Decreased	36, 37
	Morphine	CPP	Develop robust and enhanced CPP	36
βarrestin1-KO mice	Morphine	Locomotor activity	No change	37
GRK3-KO mice	Morphine	CPP	No change	19
GRK5-KO mice	Morphine	CPP	Did not develop CPP	19
MOR S375A mice	Morphine	CPP	No change	19
Phosphorylation deficient C terminus of MOR mice	Morphine	Locomotor activity	No change	21
	Fentanyl	Locomotor activity	No change	21
	Morphine	CPP	No change	21
	Fentanyl	CPP	No change	21

Subcutaneous, sc; intrathecal, it; conditioned-place preference, CPP.

TABLE S2. In Vitro Pharmacology of Compounds Compared in this Review for Biased Agonism.

	MOR Receptor Activity				
Compound	**Assay 1 G protein**	**Assay 2 βarrestin2**	**Bias Calculation Method**	**Conclusion**	**Ref**
DAMGO	GTPγS binding, cAMP accumulation	βarrestin2 enzyme complementation assay and BRET	Black-Leff operational model-no data provided*	Equal between assays	46
	GTPγS binding, cAMP accumulation	βarrestin2 enzyme complementation assay	Black-Leff operational model	Equal between assays	60
Morphine	GTPγS binding, cAMP accumulation	βarrestin2 enzyme complementation assay and BRET	Black-Leff operational model-no data provided*	Equal between assays	45
	GTPγS binding, cAMP accumulation	βarrestin2 enzyme complementation assay	Black-Leff operational model	Equal between assays	60
Herkinorin	ERK1/2 phosphorylation	βarrestin2-GFP translocation & internalization by confocal microscopy	None applied	ERK1/2 activation with poor βarrestin2 recruitment & no internalization	43
	GTPγS binding	βarrestin2-GFP translocation +/- GRK2 by confocal microscopy	None applied	G protein with no βarrestin2 translocation to the plasma membrane even when GRK2 is overexpressed	45
	cAMP accumulation +GRK2	βarrestin2 BRET +GRK2	Black-Leff operational model-no data provided*	Modest G protein bias with βarrestin2 recruitment	46
Oliceridine	cAMP accumulation	βarrestin2 enzyme complementation assay	Equiactive comparison model	G protein bias	48
	GTPγS binding, cAMP accumulation	βarrestin2 enzyme complementation assay and BRET	Black-Leff operational model-no data provided*	G protein with undetectable βarrestin2 enzyme complementation or BRET	46
	cAMP accumulation +GRK2	βarrestin2 BRET +GRK2	Black-Leff operational model-no data provided*	G protein bias	46
PZM21	GTPγS binding, cAMP accumulation	βarrestin2 enzyme complementation assay and BRET	Black-Leff operational model-no data provided*	G protein with undetectable βarrestin2 enzyme complementation or BRET	46
	cAMP accumulation +GRK2	βarrestin2 BRET +GRK2	Black-Leff operational model-no data provided*	G protein bias	46
Mitragynine	GTPγS binding	βarrestin2 enzyme complementation assay	None applied	G protein bias	58
SR-14968	GTPγS binding, cAMP accumulation	βarrestin2 enzyme complementation assay	Black-Leff operational model	G protein bias	60
SR-17018	GTPγS binding, cAMP accumulation	βarrestin2 enzyme complementation assay	Black-Leff operational model	G protein bias	60

**The methods state using the Black and Leff Operational model to compare bias, but no values or calculations are provided in the text. In cases where efficacy of the response is low, the manuscript (46) states that the data were not fit to the model.*
GTPγS binding refers to 35S-GTPγS binding in membranes.
βarrestin2 enzyme complementation assay (EFC) is the DiscoveRx PathHunter® assay.
BRET is a Bioluminescence Resonance Energy Transfer assay

TABLE S3. In Vivo Responses to Biased Agonists.

Antinociception and Tolerance

Compound	Test Subject	Model	Assay	Effect Relative to Morphine	Ref
Herkinorin	Rats	Inflammation pain	Acute flinch response in the paw formalin test	Enhanced potency but localized antinociception	44
			Tolerance flinch response in the paw formalin test	Decreased effect	44
Oliceridine	Mice	Central Antinociception	Hot plate latency	Enhanced potency with faster onset for antinociception	48
		Spinal reflex Antinociception	Tail flick latency	Enhance potency	49
			Tail flick tolerance	Decreased effect	49, 51
		Nociceptive sensitization	Opioid-induced hyperalgesia (OIH)	Decreased effect	51
	Rats	Central Antinociception	Hot plate latency	Enhance potency	48
			Hot plate latency	Similar effect (compared to oxycodone)	50
		Spinal Antinociception	Tail flick latency	Enhance potency	48
		Post-operative pain	Hindpaw incisional model	Enhance potency	48
	Human clinical trials	Opioid analgesia	Cold-pain test	Enhanced analgesia with fast onset and similar duration	52
		Post-operative pain	Average pain scores	Similar effect	54
			Treatment responders using categorical and numeric rating scale (NRS) and drug demand using patient-controlled-analgesia (PCA) over 48 hours	Similar analgesic effect with fast onset and similar duration	56
			Treatment responders using categorical and numeric rating scale (NRS) and drug demand using patient-controlled-analgesia (PCA) over 24 hours	Similar analgesic effect with fast onset	55
PZM21	Mice	Central Antinociception	Hot plate latency (affective)*	Decreased at equal dose	46
			Hot plate latency	Similar	57
			Hot plate tolerance	Similar	57
		Spinal reflex Antinociception	Tail flick latency	No Response	46
		Inflammation pain	Acute lick response in the paw formalin test	Similar	46
Mitragynine and derivatives	Mice	Central Antinociception	Hot plate latency	Similar effect	58
		Spinal reflex Antinociception	Tail flick latency	Mitragynine showed decreased potency while its derivatives displayed enhanced potency with shorter duration.	58
		Antinociception Tolerance	Not specified	Mitragynine derivative showed slower development of tolerance	58
SR-14968	Mice	Central Antinociception	Hot plate latency	Enhanced potency	60
		Spinal reflex Antinociception	Tail flick latency	Enhanced potency	60
	Rats	Spinal reflex Antinociception	Tail flick latency	Enhanced potency	63
SR-17018	Mice	Central Antinociception	Hot plate latency	Similar effect	60
			Hot plate tolerance	Decreased effect	62

Adverse Side Effects

Oliceridine	Mice	Constipation	Accumulation of fecal boli	Decreased constipation	48
			Accumulation of fecal boli	Similar	49

			Small intestinal transit - orally administered charcoal meal	Less transit delay	48
			Large intestine transit - colonic bead expulsion	Less transit delay	48
			Large intestine transit - colonic bead expulsion	Similar	49
		Physical dependence and withdrawal	Naloxone-precipitated withdrawal	Similar effect but to a lesser extent	51
	Rats	Respiratory function	Respiratory suppression	Decreased effect	48
		Abuse liability	Intracranial self-stimulation	Similar effect	49
			Self-administration	Similar effect (compared to oxycodone instead of morphine)	50
	Human clinical trials	Respiratory function	Magnitude, duration, and frequency of depressed ventilatory response to hypercapnia	Similar effect but transient	52
			Hypoventilation and respiratory suppression	Decreased effect	54
			Respiratory safety burden (RSB) using zero-inflated gamma mixture model	Decreased effect	56
			Respiratory safety burden (RSB) using zero-inflated gamma mixture model	Similar effect	55
		Gastrointestinal adverse events (AEs)	Drug Effects Questionnaire (DEQ)	Decreased and less severe effect	52
			Upper gastrointestinal effects	Decreased and less severe effect	54
			Gastrointestinal adverse effects (AEs)	Dose-related AEs but less frequent and less severe	56
			Gastrointestinal adverse effects (AEs)	Dose-dependent AEs but less frequent	55
		Abuse liability	Drug Effects Questionnaire (DEQ)	Similar but with greater therapeutic index	52
	Human clinical trials	Respiratory function	Magnitude, duration, and frequency of depressed ventilatory response to hypercapnia	Similar effect but transient	52
			Hypoventilation and respiratory suppression	Decreased effect	54
			Respiratory safety burden (RSB) using zero-inflated gamma mixture model	Decreased effect	56
PZM21	Mice	Respiratory suppression	Whole-body plethysmography	Decreased effect	46
			Whole-body plethysmography	Similar effect	57
		Respiratory suppression tolerance	Whole-body plethysmography	Not acquired (similar to morphine in this study)	57
		Reward	Conditioned-place preference (CPP)	No CPP	46
		Opioid-induced psychomotor effect	Open-field locomotor activity	Decreased effect	46
Mitragynine and derivatives	Mice	Constipation	Small intestinal transit - orally administered charcoal meal	Decreased effect	58
		Respiratory suppression	%O_2 pulse oximeter system	Decreased effect	58
		Reward	Conditioned-place preference (CPP) or aversion (CPA)	Unclear CPP or CPA	58
SR-14968	Mice	Respiratory suppression	%O_2 pulse oximeter system	Decreased effect	60
	Rats	Abuse-liability	Discriminative stimulus effects	Similar effect but with enhanced potency and efficacy ratios to produce antinociception over abuse-related discriminative stimulus effects	63
SR-17018	Mice	Respiratory suppression	%O_2 pulse oximeter system	Decreased effect	60
		Dependence and withdrawal	Assessment of somatic signs from SR-17018 withdrawal	Faster recovery	62

**PZM21 affective response to hot plate assay.*