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Atypical opioid receptors: unconventional biology and therapeutic opportunities

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ABSTRACT

Endogenous opioid peptides and prescription opioid drugs modulate pain, anxiety and stress by activating four opioid receptors, namely μ (mu, MOP), δ (delta, DOP), κ (kappa, KOP) and the nociceptin/orphanin FQ receptor (NOP). Interestingly, several other receptors are also activated by endogenous opioid peptides and influence opioid-driven signaling and biology. However, they do not meet the criteria to be recognized as classical opioid receptors, as they are phylogenetically distant from them and are insensitive to classical non-selective opioid receptor antagonists (*e.g.* naloxone). Nevertheless, accumulating reports suggest that these receptors may be interesting alternative targets, especially for the development of safer analgesics. Five of these opioid peptide-binding receptors belong to the family of G protein-coupled receptors (GPCRs)—two are members of the Mas-related G protein-coupled receptor X family (MrgX1, MrgX2), two of the bradykinin receptor family (B_1, B_2), and one is an atypical chemokine receptor (ACKR3). Additionally, the ion channel N-methyl-D-aspartate receptors (NMDARs) are also activated by opioid peptides. In this review, we recapitulate the implication of these alternative receptors in opioid-related disorders and discuss their unconventional biology, with members displaying signaling to scavenging properties. We provide an overview of their established and emerging roles and pharmacology in the context of pain management, as well as their clinical relevance as alternative targets to overcome the hurdles of chronic opioid use. Given the involvement of these receptors in a wide variety of functions, including inflammation, chemotaxis, anaphylaxis or synaptic transmission and plasticity, we also discuss the challenges associated with the modulation of both their canonical and opioid-driven signaling.

Contents

Abbreviations: ACKR3, atypical chemokine receptor 3; ATCH, adrenocorticotropic hormone; B_1, bradykinin receptor 1; B_2, bradykinin receptor 2; BAM22, bovine adrenal medulla peptide 22; DOP, delta-opioid receptor; DRG, dorsal root ganglion; GPCR, G protein-coupled receptor; IUPHAR, International Union of Basic and Clinical Pharmacology; KOP, kappa-opioid receptor; MOP, mu-opioid receptor; MrgX1, Mas-related G protein-coupled receptor X1; MrgX2, Mas-related G protein-coupled receptor X2; NMDAR, N-methyl-D-aspartate receptor; NOP, nociceptin opioid receptor; OR, opioid receptor; PAMP, proadrenomedullin N-terminal peptide; SAR, structure-activity relationship.

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1. Introduction

1.1. Discovery and nomenclature of classical opioid receptors

Ancient writings and archeological findings have dated the use of opium (from "opos", Greek for juice) as far back as antiquity. During the 19th century, morphine (named after Morpheus, the Greek god of dreams) and codeine (after "kodeia", or poppy head in Greek) were isolated from the opium poppy and later extracted in large amounts after finding evidence of the plant's analgesic properties in the treatment of postoperative pain (Hamilton & Baskett, 2000). A tremendous effort to understand the mechanisms involved in analgesia led to the discovery of three pharmacologically distinct receptors, initially proposed as μ, κ, and δ (Chen, Mestek, Liu, Hurley, & Yu, 1993; Evans, Keith Jr., Morrison, Magendzo, & Edwards, 1992; Gilbert & Martin, 1976; Martin, 1979; Zhu et al., 1995). Parallel to this endeavor, the first endogenous opioid receptor ligands Met- and Leu-enkephalin were identified (Hughes et al., 1975), which grounded the hypothesis that opioid drugs relied on the modulation of an endogenous opioid machinery, with its own physiological relevance. The synthesis of naloxone, a morphinan derivative opioid antagonist, was crucial in further characterizing the opioid receptor (OR) family. Its nonselective antagonism was later used as the inclusion criterion in the pursuit of new opioid receptors. However, this rule was bent for the more recently discovered naloxone-insensitive nociceptin/orphanin FQ receptor, NOP (Mollereau et al., 1994), due to its high structural and functional homology to classical opioid receptors (Nothacker et al., 1996). It is hence classified by the International Union of Basic and Clinical Pharmacology (IUPHAR) as a fourth member of the classical opioid receptor family, joining the mu, kappa and delta opioid receptors (also named μ (MOP), κ (KOP), and δ (DOP) receptors) (Cox, Christie, Devi, Toll, & Traynor, 2015) (Fig. 1).

The pharmacological profiles of opioid receptors were characterized alongside the discovery of opioid peptide precursors, namely: proenkephalin, pro-opiomelanocortin, prodynorphin and pronociceptin (Fig. 2A). Each receptor typically shows a preference for one opioid

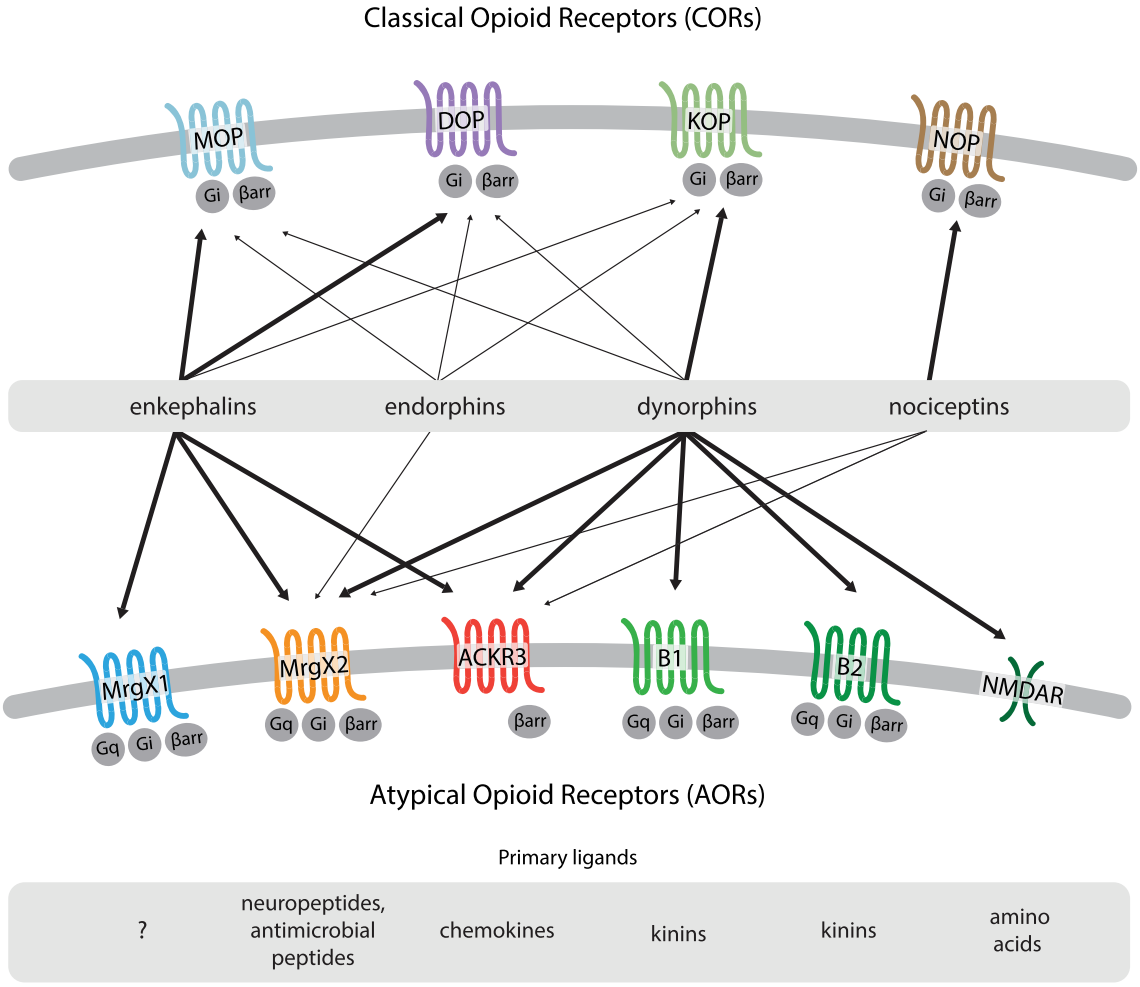


Fig. 1. Schematic representation of the interaction network of the four classical opioid receptors (CORs) and six proposed atypical opioid receptors (AORs) with their respective endogenous ligands and their intracellular interaction partners: G proteins and β-arrestins. Receptor colors reflect their preference for ligand families: enkephalins - blue/violet, dynorphins - green and nociceptin - brown. The more promiscuous atypical opioid receptors, MrgX2 and ACKR3, are colored orange and red. The width of arrows reflects the strength of ligand–receptor interactions.

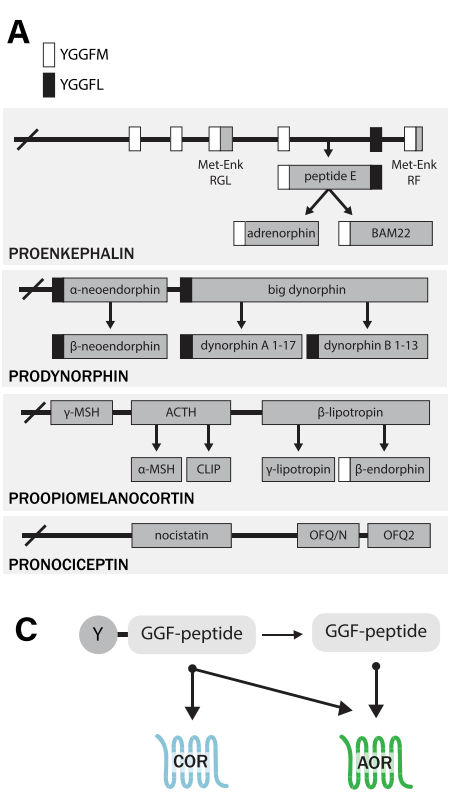
peptide family and some cross-selectivity for another (Gomes et al., 2020). MOP binds preferentially enkephalins and endorphins, DOP displays high affinity for Met- and Leu-enkephalin and KOP binds prodynorphin-derived products. All three receptors show some degree of cross-family selectivity, while NOP binds exclusively nociceptin (also known as orphanin FQ, abbreviated OFQ/N) and nociceptin-derived ligands (Fig. 1). Structure-activity relationship (SAR) studies of the cleaved products of these precursors were reviewed extensively for MOP, DOP, KOP and NOP (Henderson & McKnight, 1997; Janecka, Fichna, & Janecki, 2004). It has been proposed that endogenous opioid peptides have two specific recognition regions, namely the N-terminal "message" containing residues YGGF, and the "address" made up of the C-terminal residues (Mansour, Hoversten, Taylor, Watson, & Akil, 1995). These are key determinants of classical opioid receptor activation and selectivity, respectively. While the YGGFL/M sequence is necessary and sufficient for MOP and DOP activation, KOP requires the longer YGGFL/MRR/K sequence (Mansour et al., 1995). Of note, the precursors of endomorphin-1 and endomorphin-2, two peptides with a high affinity and selectivity towards the MOP (Fichna, Janecka, Costentin, & Do Rego, 2007; Hackler, Zadina, Ge, & Kastin, 1997) that do not share the YGGF motif, have yet to be found in the human proteome, leading to a degree of controversy regarding their endogenous existence (Terskiy et al., 2007).

All four opioid receptors are class A G protein-coupled receptors (GPCRs) involved in a variety of physiological and pathophysiological events, including but not limited to pain modulation, immune function and emotional response. Upon opioid peptide binding, classical ORs activate heterotrimeric αβγ G proteins, leading to Gα-Gβγ dissociation and $G_{\alpha i/o}$-dependent inhibition of adenylyl cyclase and subsequent decrease of intracellular cAMP levels. In postsynaptic neurons, the dissociated $G_{\beta\gamma}$ dimer activates G protein-coupled inwardly-rectifying potassium channels (GIRKs), causing hyperpolarization and inhibition of neurons, while presynaptically, it inhibits voltage-gated calcium channels (VGCC) to prevent the release of neurotransmitters. Moreover, classical opioid receptors are able to induce the activation of several downstream kinases, including the extracellular signal-regulated kinases 1 and 2 (ERK1 and 2) of the mitogen-activated protein kinase (MAPK) cascade (Al-Hasani & Bruchas, 2011). Ultimately, G protein-coupled receptor kinases (GRKs) phosphorylate activated receptors, thereby promoting β-arrestin recruitment, which eventually leads to receptor desensitization and internalization (Al-Hasani & Bruchas, 2011; Connor & Christie, 1999).

1.2. Classical OR modulation for pain treatment and its limitations

Opioid drugs have proven exquisitely powerful against moderate to severe degrees of acute and chronic pain and are among the most commonly used analgesics in the clinic (Melnikova, 2010). They are typically MOP agonists, with morphine standing at the forefront, along with fentanyl and oxycodone (Hider-Mlynarz, Cavalie, & Maison, 2018; Monje, Gimenez-Manzorro, Ortega-Navarro, Herranz-Alonso, & Sanjurjo-Saez, 2019).

However, while opioid drugs are instrumental in pain management, their use in some applications is hindered by a limited benefit/risk ratio.



B

precursor	ligand	sequence	MOP	DOP	KOP	NOP	ACKR3	MrgX1	MrgX2	B_1	B_2	NMDAR
			classical opioid receptors				atypical opioid receptors					
proenkephalin	[Met]enkephalin	YGGFM	●	●	●	■	■	■	■			
	adrenorphin	YGGFMRRV-NH_2	●	●	●	■	●					
	BAM18	YGGFMRRVGRPEWWMDYQ	●	●	●	■	●					
	BAM22	YGGFMRRVGRPEWWMDYQKRYG	●	●	●	■	●	●	■			
	BAM8-22	- - - - - - - - VGRPEWWMDYQKRYG	■	■	■	■	■	●	●			
	peptide E	YGGFMRRVGRPEWWMDYQKRYGGFL	●	●	●	■	●	●				
prodynorphin	dynorphin A 1-17	YGGFLRRIRPKLKWDNQ	●	●	●	■	●	■	●	●	●	●
	dynorphin A 1-13	YGGFLRRIRPKLK	●	●	●	■	●	■	●			●
	dynorphin A 2-13	- GGFLRRIRPKLK	■	■	■	■	●			●	●	●
	dynorphin A 2-17	- GGFLRRIRPKLKWDNQ	■	■	■	■	●	■				●
	dynorphin B	YGGFLRRQFKVVT	●	●	●	■	●		●			■
	leumorphin	YGGFLRRQFKVVTRSQEDPNAYSGELFDA	●	●	●	■	●					
	big dynorphin	YGGFLRRIRPKLKWDNQKRYGGFLRRQFKVVT	●	●	●	■	●					●
	α-neoendorphin	YGGFLRKYRPK	●	●	●	■	■		●			
	β-neoendorphin	YGGFLRKYP	●	●	●	■	■		●			
	[Leu]enkephalin	YGGFL	●	●	■	■	■	■	■			■
proopio-melanocortin	α-endorphin	YGGFMTSEKSQTPLVT										
	β-endorphin	YGGFMTSEKSQTPLVTLFKNAIIKNAYKKGE	●	●	●	■	■		●			
	γ-endorphin	YGGFMTSEKSQTPLVTL					■					
pronociceptin	nociceptin	FGGFTGARKSARKLANQ	■	■	■	●	●	■	●			
	nociceptin 1-13	FGGFTGARKSARK-NH_2	■	■	■	●	●					
references				(1) (2) (3)		(1) (3) (4)	(3) (5)	(6) (7)	(8)	(9) (10)		(11) (12) (13) (14)

● interaction ■ no interaction

Fig. 2. Opioid peptide maturation and interactome. A) Biosynthesis and processing of endogenous opioid peptides from proenkephalin, prodynorphin, pro-opiomelanocortin and pronociceptin precursors. B) Overview of the reported interactions of endogenous opioid and opioid-derived peptides on atypical opioid receptors and comparison with classical opioid receptors. Peptides are grouped by their common precursors. The peptides with a truncated N-terminal YGGF motif are highlighted in grey. Green indicates reported interaction, red indicates reported lack of interaction. Of note, the interactions reported were identified using various techniques, including binding competition, β-arrestin recruitment, Ca^{2+} flux assays, etc., hence, no comparison of potencies should be drawn. Information was extracted from the following references: (1) Janecka et al., 2004; (2) Mansour et al., 1995; (3) Meyrath et al., 2020; (4) Gomes et al., 2020; (5) Henderson & McKnight, 1997; (6) Ikeda et al., 2013; (7) Lembo et al., 2002; (8) Burstein et al., 2006; (9) Lansu et al., 2017; (10) Lai et al., 2006; (11) Lee et al., 2014; (12) Chen et al., 1995; (13) L. Chen & Huang, 1998; (14) Tan-No et al., 2002; (15) Tang et al., 1999. C) Full-length endogenous opioid peptides are active on classical (COR) and atypical (AOR) opioid receptors, while peptides with a truncated N-terminal YGGF motif bind atypical ORs and are inactive on classical ORs.

Studies comparing a panel of opioids in patient-controlled analgesia have pointed to various side effects comprising nausea/vomiting, pruritus, respiratory depression, decreased gastrointestinal motility and addiction (Dinges et al., 2019; Lutz & Kieffer, 2013). The latter is largely responsible for the current opioid crisis, which dates back to the 1990s and refers to the dramatic rise in opioid use and overdose deaths until now (CDC and N. C. f. H. S, 2021) https://www.cdc.gov/drugoverdose/data/. Furthermore, long-term opioid treatment is associated with rapid tolerance, *i.e.* the requirement to escalate doses to obtain equivalent analgesia, simultaneously aggravating aforementioned adverse effects (Bailey & Connor, 2005; Buntin-Mushock, Phillip, Moriyama, & Palmer, 2005; Dumas & Pollack, 2008; Morgan & Christie, 2011). This implies that repeated opioid administration may quickly lead to unwanted side effects overstepping pain relief, which in part justifies that current CDC guidelines only encourage sustained opioid therapy to manage chronic cancer pain, palliative and end-of-life care, while non-opioid therapy should prevail in the management of non-cancer chronic pain conditions (Dowell, Haegerich, & Chou, 2016).

Such a climate warrants the search for opioid drugs with different pharmacological profiles that exploit the analgesic potential of opioid receptors, while limiting the onset of associated adverse events.

Indeed, a more favorable adverse effect profile was thought to be partly addressed with the discovery of "functional selectivity" or "biased signaling", which refers to the ability of a ligand to preferentially activate one pathway over another (Smith, Lefkowitz, & Rajagopal, 2018). This concept generated remarkable enthusiasm for the discovery of molecules with pharmacological profiles which could offer more control over GPCR modulation, leading to a substantial body of studies aiming at deciphering the contribution of single effector proteins towards one phenotype. Biased signaling has been extensively investigated at the MOP (Bohn et al., 1999; Raehal, Walker, & Bohn, 2005), the main GPCR targeted by synthetic opioids used in the clinic, but also the KOP (Bruchas et al., 2007; Bruchas, Macey, Lowe, & Chavkin, 2006; Chavkin, Schattauer, & Levin, 2014) and the DOP (recently reviewed by (Pineyro & Nagi, 2021)). All of the above studies advocate that G protein activation is responsible for opioid-induced analgesia, while β-arrestin bias enhances receptor internalization, desensitization and potentially triggers alternative signaling pathways, underlying the overall manifestation of tolerance and dependence. In this context, two MOP agonists were developed, PZM21 (Manglik et al., 2016) and TRV130 (oliceridine, recently approved by the FDA) (DeWire et al., 2013; Mullard, 2020; Singla et al., 2017), which show little β-arrestin recruitment or receptor internalization, both in cell lines and native neurons (Ehrlich et al., 2019). However, the proposed G protein bias is still a source of contention, with divergent results reported in the literature and more work is still required to evaluate this strategy at each opioid receptor subtype (Altarifi et al., 2017; Bachmutsky, Wei, Durand, & Yackle, 2021; He et al., 2021; Hill et al., 2018; Kliewer et al., 2019; Kliewer et al., 2020). Overall, these observations suggest that G protein-biased ligands may also induce side effects, stressing the urgent need to search for alternative approaches to modulate opioid receptors.

Positive allosteric modulators (PAMs) of the MOP that by binding to a topographically distinct site improve both the potency and efficacy of orthosteric ligands (endogenous and exogenous opioids) have been described (Burford et al., 2013; Burford, Traynor, & Alt, 2015). Recent preclinical studies with one of these PAMs has shown decreased side effects and an elevation in antinociception in animal models of acute noxious heat pain and inflammatory pain (Kandasamy et al., 2021).

Importantly, the use of small molecules with a multi-targeting profile has also shown some potential to increase the benefit/risk ratio. Bivalent ligands have been developed to simultaneously target MOP-DOP (Parenti et al., 2012; Podolsky et al., 2013), MOP-KOP (Lazenka et al., 2018) and MOP-NOP (Toll et al., 2009). However, although there has been evidence of the potentiation of MOP-mediated antinociception, the use of bivalent ligands is often limited by their side effects associated with the activation of the other opioid receptor counterpart. This is discussed extensively elsewhere (Gunther et al., 2018).

Another approach to modulate the endogenous opioid system could be to interfere with other receptors that do not fulfill the initial criteria for inclusion within the classical opioid receptor family. Several receptors have been reported to bind endogenous opioid peptides despite their insensitivity to naloxone and little sequence or structural homology with classical opioid receptors. The notion of atypical opioid receptor was introduced in 2017 following the finding that prodynorphin-derived peptides can bind to Mas-related G protein-coupled Receptor-X2 (MRGPRX2 or MrgX2), a GPCR initially known for its role in mast cell degranulation (Lansu et al., 2017). A second member of this family, MrgX1, as well as the family of bradykinin receptors (B_1 and B_2) and the ligand-gated ion channel N-methyl-D-aspartate receptors (NMDARs) have also been shown to respond to endogenous opioids such as enkephalins or dynorphins (Chen, Gu, & Huang, 1995; Chen & Huang, 1998; Lai et al., 2006; Lembo et al., 2002; Massardier & Hunt, 1989). Recently, the atypical chemokine receptor ACKR3 (previously CXCR7) was suggested as another atypical opioid receptor, based on its unique ability to scavenge a variety of endogenous opioid peptides without inducing canonical downstream G protein-mediated signaling (Meyrath et al., 2020) (Table 1).

These receptors, which have defined functions in a variety of responses, including inflammation, synaptic transmission, anaphylaxis or chemotaxis, have shown clear evidence of modulation of the opioid system. They may therefore provide an alternate route to opioid-mediated analgesia through their direct activation or indirect modulation of classical ORs, which might also apply to chronic pain management.

2. Atypical opioid receptors - unconventional biology

2.1. Mas-Related Receptors GPCR member X (MRGX)

The MRGX family is made up of MRGPRX1, MRGPRX2 (abbreviated MrgX1 and MrgX2), MRGPRX3 and MRGPRX4. Currently, all four members are classified as orphan receptors by the IUPHAR (Davenport et al., 2013) although endogenous ligands of varying affinities have been proposed for MrgX1 and MrgX2, and more recently for MrgX4 (Meixiong, Vasavda, Snyder, & Dong, 2019; Yu et al., 2019). Their major function is recognized to be pruriception as they typically bind itch-inducing compounds, with relative promiscuity (Bader, Alenina, Andrade-Navarro, & Santos, 2014).

Human and rhesus MrgX1 and MrgX2 display a high activity in proliferation assays, which is partially blocked by pertussis toxin, indicative of $G_{i/o}$ coupling. Both of these receptors have been found to increase intracellular Ca^{2+} levels through a $G_{q/11}$ coupling as well (Burstein et al., 2006) (Fig. 1).

MrgX1 was initially coined sensory neuron-specific receptor 4 (SNSR4) due to its unique localization, restricted to the dorsal root ganglia (DRG) and trigeminal ganglia. It binds the proenkephalin A-derived bovine adrenal medulla peptide BAM22 but also the N-terminally truncated form BAM8-22, its most potent endogenous ligand found to date (Lembo et al., 2002) (Fig. 2B). Activation of MrgX1 leads to inhibition of high-voltage-activated Ca^{2+} channels, involved in noxious transmission (Li et al., 2014). Its contribution to neuropathic pain and itch has been characterized in multiple models using BAM8-22 (He et al., 2014; Liu et al., 2009). Furthermore, its expression restricted to the peripheral nervous system (PNS) makes it a potential target to treat chronic pain without the adverse events accredited to the central nervous system (CNS). Although MrgX1 binds the endogenous enkephalin BAM22, this interaction does not depend on its N-terminal YGGF motif since it is also able to bind the truncated BAM8-22, highlighting that the receptor binding mechanism is distinct from classical ORs.

The second MrgX family member MrgX2 was described in 2003 as a cortistatin receptor, and was later found to be activated by

Table 1
Summary of referenced studies reporting an interaction between opioid and opioid-derived peptides with atypical opioid receptors and related effects

Receptor	Active peptides	*In vitro* observation	*In vivo* observation	Reference
MrgX1	BAM22 and various processed forms, including BAM8-22	[stably expressing HEK293s cells] Concentration-dependent release of intracellular calcium Whole-cell binding analysis and competition binding using [^{3}H] BAM8–22 as tracer		Lembo et al., 2002
	BAM22, BAM8-22	[stably and transiently expressing HEK293T cells] Concentration-dependent release of intracellular calcium Concentration-dependent increase of GTP-γS binding Constitutive and agonist-induced proliferative response		Burstein et al. 2006
	BAM8-22	[transiently expressing HEK293T cells] Concentration-dependent release of intracellular calcium Induction of action potentials in whole-cell patch clamp recordings	Intradermal injection of BAM8-22: stronger induction of itch response in WT than *Mrgpr-cluster*$^{\Delta-/-}$ mice	Liu et al., 2009
	BAM8-22	[transiently expressing HEK293T cells] Concentration-dependent release of intracellular calcium	[MrgC - rodent homolog to MrgX1] Intrathecal injection of BAM8-22: antinociceptive effect in rodent models of neuropathic pain (spinal nerve ligation, SNL)	He et al., 2014
	BAM8-22	[acutely dissociated DRG neurons from MrgprX1 mice] Concentration-dependent inhibition of calcium channels [substantia gelatinosa neurons in lumbar spinal cord slices] Inhibition of spinal synaptic transmission	Intrathecal administration of BAM8-22: inhibition of neuropathic pain-related behavior in *MrgprX1* but not *Mrgpr*$^{-/-}$ mice	Li et al., 2017
MrgX2	BAM22	[transiently expressing HEK293T cells] Concentration-dependent release of intracellular calcium Constitutive and agonist-induced proliferative response		Buntin-Mushock et al., 2005
	dynorphin A and truncated forms, dynorphin B, α-neoendorphin, BAM22, BAM8-22	[MrgX2-inducible stable HEK293T cells] Concentration-dependent release of intracellular calcium [LAD2 mast cell line] Induction of degranulation		Lansu et al., 2017
ACKR3	various enkephalins, mainly BAM22 and related peptides	[stably and transiently expressing HEK293 cells and NCI-H295R adrenocortical cells] Recruitment of β-arrestin1 and β-arrestin2 Binding competition with [^{125}I]-CXCL12 Increase in ACTH-induced ERK phosphorylation Enhanced cortisol secretion	Subcutaneous injection of ACKR3 agonist, anxiolytic-like behavior and increase of circulating glucocorticoid levels	Ikeda et al., 2013
	various enkephalins, dynorphins, nociceptins and truncated forms	[stably and transiently expressing HEK293, U87, CHO cells, and small molecule neural precursor cells (smNPCs)] Recruitment of β-arrestin1 and β-arrestin2 Binding competition with fluorescently labeled CXCL12 Uptake of fluorescently labeled opioids Agonist-induced receptor disappearance from cell surface and transport into endosomal compartments Depletion of opioid peptides in extracellular space	Increased opioid peptide-mediated neuronal firing after ACKR3 inhibition in (*ex vivo*) rat locus coeruleus tissue	Meyrath et al., 2020
	dynorphin A, nociceptin 1-13, BAM22	[transiently expressing U87 cells] Recruitment of β-arrestin1 and β-arrestin2 Agonist-induced receptor disappearance from cell surface and transport into endosomal compartments Binding and uptake of fluorescently labeled BAM22 Binding competition with fluorescently labeled CXCL12		Szpakowska et al., 2021

(continued on next page)

Table 1 (*continued*)

Receptor	Active peptides	*In vitro* observation	*In vivo* observation	Reference
B1	dynorphin A, dynorphin A 2-13	[B1-transiently transfected F-11 cells] Concentration-dependent release of intracellular calcium Binding competition with [^{3}H]kallidin	Intrathecal injection of dynorphin A 2-13 in rats: increase in tactile hypersensitivity and thermal hyperalgesia Partial reversal of dynorphin A 2-13-induced thermal hyperalgesia after treatment with B1-specific inhibitor DALBK Partial reversal of L5-L6 spinal nerve ligation hypersensitivities after spinal administration of B1-specific antagonist DALBK	Lai et al., 2006
	dynorphin A		Model of inflammatory pain with spinal upregulation of dynorphin A Intrathecal administration of B1 antagonist DALBK: reversal in thermal and tactile hypersensitivities, in an equivalent measure as treatment with anti-dynorphin antiserum	Luo et al., 2008
	dynorphin A and mutated forms, including dynorphin A 2-13	[rat brain membranes or transiently expressing HEK293 cells] Binding competition using [^{3}H]DALKD or [^{3}H]BK		Lee et al., 2014
B2	dynorphin A, dynorphin A 2-13	[endogenously or transiently expressing F-11 cells and primary cultures of dorsal root ganglia] Concentration-dependent release of intracellular calcium, inhibited with B2-specific antagonist HOE 140 [endogenously or transiently expressing F-11 or COS-7 cells, and mouse whole-brain membranes] Binding competition with [^{3}H]bradykinin	Intrathecal injection of dynorphin A 2-13 in rats: increase in tactile hypersensitivity and thermal hyperalgesia Partial reversal of dynorphin A 2-13-induced thermal hyperalgesia after treatment with B2-specific inhibitor HOE 140 Absence of hypersensitivity following dynorphin A 2-13 intrathecal injection in *Bdkrb2*-KO mice Partial reversal of L5-L6 spinal nerve ligation hypersensitivities after spinal administration of B2-specific antagonist HOE 140	Lai et al., 2006
	dynorphin A		Model of inflammatory pain with spinal upregulation of dynorphin A Intrathecal administration of B2 antagonist HOE 140: reverse in thermal and tactile hypersensitivities, in an equivalent measure as treatment with anti-dynorphin antiserum	Luo et al., 2008
	dynorphin A and mutated forms, including dynorphin A 2-13	[rat brain membranes or transiently expressing HEK293 cells] Binding competition with [^{3}H]DALKD or [^{3}H]BK	Pretreatment with specific B2 inhibitor blocks dynorphin A 2-13-induced paralysis, thermal hyperalgesia and mechanical hypersensitivity	Lee et al., 2014
NMDAR	dynorphin A 1-13	[membrane preparation from rat cortex] Binding competition with L-[^{3}H]glutamate or [^{3}H]MK-801		Massardier & Hunt, 1989
	dynorphin A, dynorphin A 1-13, dynorphin A 2-17, big dynorphin	[acutely dissociated trigeminal neurons in rat] Recording of whole-cell and single-channel currents Concentration-dependent reduction of NMDA-activated currents		Chen et al., 1995
	dynorphin A, dynorphin A 1-13 and further C-terminally truncated variants	[acutely dissociated trigeminal neurons in rat] Recording of whole-cell and single-channel currents Concentration-dependent reduction of NMDA-activated currents		Chen & Huang, 1998
	dynorphin A 2-17	[membrane preparation from rat cortex and transiently transfected HEK293 cells with NR1a/NR2a subunits of NMDAR] Binding competition and saturation with ^{125}I- dynorphin A 2-17		Tang et al., 1999
	big dynorphin		big dynorphin-induced nociceptive behavior in mice Intrathecal co-administration of NMDAR antagonists D-APV, MK-801 or Ifenprodil: dose-dependent induction of antinociceptive behavior	Tan-No et al., 2002
	Big dynorphin		Injection of big dynorphin inducing locomotor activity, memory and anxiolytic-like behavior in mice Intracerebroventricular co-administration with NMDAR antagonist MK-801: dose-dependent inhibition of aforementioned behavioral effects	Kuzmin et al., 2006

proadrenomedullin N-terminal 20 peptide (PAMP-20) and its truncated analog PAMP-12 (PAMP[9-20]) (Kamohara et al., 2005; Robas, Mead, & Fidock, 2003). It is highly expressed in mast cells, the primary effectors in anaphylaxis, and is responsible for most pseudo-allergic drug reactions. A large variety of FDA-approved peptidergic drugs elicit an injection-site reaction associated with pain and itch, which has now been attributed to MrgX2 promiscuous activation by cationic peptides (McNeil et al., 2015). MrgX2 activation following morphine exposure was confirmed at clinically relevant doses (Navines-Ferrer et al., 2018).

More recently, MrgX2 was shown to respond to micromolar concentrations of prodynorphin-derived peptides and synthetic opioids displaying an uncharacteristic preference for dextromorphinans and dextrobenzomorphans (Lansu et al., 2017) (Figs. 1 and 2). MrgX2 is expressed in the PNS and CNS, with highest levels in DRG, where it may act in competition or synergy with MOP, DOP and KOP.

2.2. Bradykinin Receptors (BRs)

There are two members of the bradykinin receptor (BR) family, bradykinin receptor 1 (B_1) and 2 (B_2). Their pharmacological and expression profiles are widely different: B_1 is expressed in many immune cell types upon induction by proinflammatory cytokines and undergoes limited desensitization, while B_2 is constitutively expressed in the PNS and extensively desensitized (Bertram et al., 2007; Kawaguchi et al., 2015; Leeb-Lundberg, Marceau, Muller-Esterl, Pettibone, & Zuraw, 2005; Medeiros et al., 2004). Both receptors bind with high affinity to different cleaved fragments of kininogen precursors to mediate inflammation, angiogenesis and vasodilatation.

B_1 binds the endogenous bradykinin and Lys-[des-Arg^9]-bradykinin with nanomolar affinity. Its expression and signaling following tissue injury induces the recruitment of neutrophils and activation of Ca^{2+}-mediated nitric oxide synthases, which further regulate the inflammatory responses and vascular tone (Dhamrait et al., 2003; Ehrenfeld et al., 2006). B_2 is activated by bradykinin and kallidin (also Lys-bradykinin), and induces MAPK phosphorylation via transactivation of epidermal growth factor receptor (EGFR) (Vidal et al., 2005). B_2 is responsible for the majority of bradykinin-mediated responses. Both receptors couple to $G_{i/o}$ and $G_{q/11}$ synergistically, inhibiting adenylyl cyclase and inducing intracellular Ca^{2+} mobilization (Fig. 1).

Bradykinin was first linked to nociception over three decades ago, when it was observed to lead to PKC-mediated depolarization and subsequent sensitization to noxious stimuli in neonatal rat DRG neurons (Burgess, Mullaney, McNeill, Dunn, & Rang, 1989). Consistently, bradykinin antagonists alleviate hyperalgesia in rat models of acute and chronic pain (Steranka et al., 1988).

In 2006, dynorphin A was reported to activate both bradykinin receptors, promoting inflammation and hyperalgesia (Lai et al., 2006; Luo et al., 2008). Importantly, radioligand binding studies revealed that Dyn A (2-13), a truncated variant of dynorphin A, also competed with bradykinin for B_2 (Lai et al., 2006; Lee et al., 2014). It should be noted that, this variant was only shown to activate bradykinin receptors, NMDARs and ACKR3, and despite its lack of affinity towards classical ORs, it seems to have important implications in opioid-related phenotypes, including learning capacity and memory (Hiramatsu & Inoue, 2000).

Although dynorphin A is structurally different from previously identified ligands of BRs, their interaction has been validated by multiple groups (Lai et al., 2006; Lai, Luo, Chen, & Porreca, 2008; Lee et al., 2016; Luo et al., 2008). As a family of receptors which has been implicated in the perception of pain with their prototypical ligands, BRs may also modulate nociception through their interaction with the endogenous opioid peptide dynorphin A and its derived truncated forms.

2.3. Atypical chemokine receptor ACKR3/CXCR7

The atypical chemokine receptor 3 (ACKR3, formerly known as RDC-1 and CXCR7) was deorphanized in 2005 with the identification of chemokines CXCL12 (SDF-1) and CXCL11 (I-TAC) as its endogenous ligands, which it shares with the classical chemokine receptors CXCR4 and CXCR3, respectively (Balabanian et al., 2005; Burns et al., 2006; Szpakowska et al., 2018). ACKR3 is also a receptor for the HHV-8 encoded chemokine vCCL2/vMIP2 (Szpakowska et al., 2016; Szpakowska & Chevigne, 2016) and for proadrenomedullin N-terminal 20 peptides (PAMPs) (Meyrath et al., 2021).

Initially a member of the classical chemokine receptor family and named CXCR7, it was officially declassified as such in 2014 with the introduction of the atypical chemokine receptor (ACKR) subfamily by the IUPHAR (Bachelerie et al., 2014). This family encapsulates four receptors, highly homologous to classical chemokine receptors, which vary considerably in their biology but share the common characteristic of being unable to trigger canonical G protein signaling. Instead, ACKRs act either as buffers by temporarily capturing chemokines, or as scavengers by internalizing and degrading them (Nibbs & Graham, 2013). They shape chemokine gradients during immune responses and development, maintaining homeostasis and participating in the resolution of inflammation events.

ACKR3 in particular is expressed in the majority of CNS regions, in the adrenal glands, on a number of immune cell subsets and on endothelial cells, where it scavenges CXCL12 to modulate hematopoietic stem cell migration for instance (Koenen, Bachelerie, Balabanian, Schlecht-Louf, & Gallego, 2019; Quinn, Mackie, & Caron, 2018; Saaber et al., 2019). ACKR3 is also involved in cardiac development and $ACKR3^{-/-}$ knock-out is a perinatal lethal phenotype, with embryos showing cardiac hyperplasia and vascular defects (Sierro et al., 2007). ACKR3 also plays a key role in neuronal development by modulating the CXCR4-CXCL12 axis, which is also often exploited and upregulated in metastatic cancers (Puddinu et al., 2017; Sjoberg et al., 2020; Smit et al., 2021; Zou, Kottmann, Kuroda, Taniuchi, & Littman, 1998).

In 2013, Ikeda et al. reported ACKR3 to bind with high affinity a range of endogenous proenkephalin A-derived peptides, such as BAM22 and peptide E but not the further processed Met-enkephalin or Leu-enkephalin (Ikeda, Kumagai, Skach, Sato, & Yanagisawa, 2013) (Fig. 2B). Recently, ACKR3 selectivity for opioid peptides was extended from enkephalins to members of the dynorphin and nociceptin families (Meyrath et al., 2020) (Figs. 1 and 2). The range of potencies of many of these opioid peptides for ACKR3, evaluated with β-arrestin 1 and β-arrestin 2 recruitment, is comparable with those of classical ORs towards their prototypic ligands, clearly suggesting biological relevance of these promiscuous receptor–ligand interactions.

Interestingly, ACKR3 is highly tolerant towards N-terminal tyrosine modifications of its opioid peptide ligands. An initial mutational study revealed that BAM22 and [Phe^1]BAM22 equally activated the receptor (Ikeda et al., 2013). This was further validated by a SAR analysis on adrenorphin (YGGFMRRV-NH_2), another ligand of ACKR3 as well as MOP, DOP and KOP (Meyrath et al., 2020). Variants with a Y1F mutation lead to a tenfold increase in potency for ACKR3, while it predictably abolishes classical OR activation. This leniency for the first tyrosine residue accounts for the activation and recognition of processed opioid fragments such as dynorphin $A_{2\text{-}13}$ and dynorphin $A_{2\text{-}17}$. Hence, although ACKR3 recognizes a broad range of opioid peptides, its activation relies on determinants distinct from the classical OR recognition sites.

ACKR3 signaling is still a source of debate, within and outside the opioid context and it cannot be excluded that in specific cell types or cellular contexts, ACKR3 may induce G protein-dependent or independent signaling (Fumagalli et al., 2020; Odemis et al., 2012). β-arrestin signaling was proposed for ACKR3 in some reports (Heuninck et al., 2019; Rajagopal et al., 2010). However, little consensus has been reached regarding the existence of direct β-arrestin signaling by GPCRs in general, and the evidence supporting such signaling has been challenged repeatedly (Alvarez-Curto et al., 2016; Grundmann et al., 2018; Kliewer et al., 2019; Meyrath et al., 2020; O'Hayre et al., 2017; Smith et al., 2018).

Direct ACKR3 signaling in response to opioid peptide binding also remains contentious. ACKR3 was first reported to modulate

adrenocorticotropic hormone (ACTH)-driven intracellular responses through the ACTH–melanocortin 2 receptor (MC2R) axis. This was suggested to stem from BAM22-mediated activation of ACKR3, eventually leading to β-arrestin recruitment and an increase in ERK1/2 phosphorylation (Ikeda et al., 2013). However, recent studies could not bring evidence for such phosphorylation or any G protein interaction (Meyrath et al., 2020; Szpakowska et al., 2021). This non-signaling behavior is consistent with previous records of ACKR3 modus operandi for chemokine ligands, acting as a scavenger to deplete the microenvironment and classical receptors of their ligands (Quinn et al., 2018).

ACKR3 presence in the CNS as well as in adrenal glands puts it at the forefront of opioid peptide-mediated pathways and its activation shows downstream anxiolytic-like effects in a preclinical study (Ikeda et al., 2013). Gene expression analysis revealed that ACKR3 is highly expressed in several brain regions corresponding to important opioid activity hubs. Additionally, its expression levels are often higher than those of classical opioid receptors, which further supports the physiological relevance of its observed *in vitro* opioid peptide scavenging capacity. This is reinforced by the finding that blocking ACKR3 scavenging through administration of the modulator LIH383 leads to potentiation of dynorphin A effects on the classical opioid receptors, *i.e.* an increase in the inhibition of neuronal firing (Meyrath et al., 2020). Additionally, ACKR3 was recently shown to bind the natural analgesic molecule conolidine, further pointing to the involvement of this receptor in pain (Szpakowska et al., 2021).

ACKR3 is a well-established atypical chemokine scavenger and this function seems to extend to endogenous opioid peptides. Its nanomolar affinity for several of these families, its proposed involvement in pain and anxiety, and its insensitivity to naloxone align to make it a substantial target for non-classical opioid tone modulation.

2.4. N-Methyl-D-aspartic acid receptors (NMDARs)

The N-Methyl-D-aspartic acid receptors (NMDARs) are heterotetrameric voltage-dependent glutamate-gated ion channels with high Ca^{2+} permeability. The gating of these channels is quite complex and depends on two mechanisms *i.e.* ligand binding and membrane depolarization. Extracellular Mg^{2+} and Zn^{2+} bind to the receptor and block the passage of other cations. It requires membrane depolarization to eliminate channel inhibition and, depending on its subunit composition, glutamate or glycine/D-serine binding to allow a Ca^{2+} influx (Planells-Cases, Sun, Ferrer-Montiel, & Montal, 1993; Wolosker, 2006). NMDARs are constitutively expressed in numerous brain regions and are essential mediators of synaptic plasticity and excitatory neurotransmission (Traynelis et al., 2010).

Several reports have documented dynorphin A as a competitive ligand for glutamate on NMDARs (Massardier & Hunt, 1989; Tang et al., 1999) (Figs. 1 and 2). Interestingly, big dynorphin (Big Dyn), the intermediary precursor of dynorphin A and B, which is expressed in the brain, pituitary gland and spinal cord, shows little activity *in vivo* towards KOP, despite its high affinity for the receptor, comparable to dynorphin A and B (Kuzmin, Madjid, Terenius, Ogren, & Bakalkin, 2006; Merg et al., 2006). It may instead mediate physiological and pathological processes through NMDARs. Indeed, intrathecal administration of Big Dyn has been reported to induce naloxone-insensitive nociceptive behavior in mice, which was dose-dependently inhibited by NMDAR antagonists (Tan-No et al., 2002). Endogenous Big Dyn was also shown to induce OR-independent nociception through the use of dynorphin degradation inhibitors (Tan-No et al., 2005).

In another study evaluating KOP-associated behavioral responses, dynorphin A and B administration was associated with antinociception in the hot-plate test, whereas Big Dyn led to memory enhancing, locomotor and anxiolytic-like effects (Kuzmin et al., 2006). While the effects of dynorphin A and B were inhibited by the KOP antagonist norbinaltorphimine (norBNI), those induced by Big Dyn were insensitive to naloxone and blocked by the NMDAR antagonist MK-801. This suggests that NMDARs bind specifically to Big Dyn and modulate similar endpoints as classical ORs but with different effectors and behavioral responses.

NMDARs have been colocalized with MOP in CNS neurons, specifically in the dendrites and somata of ventrolateral PAG neurons (Commons, van Bockstaele, & Pfaff, 1999) and there are many reports of a putative interaction between the two receptors. Rodriguez-Munoz et al. suggest that the heterodimer NMDAR–MOP forms during single-dose administration of opioids (e.g. morphine, fentanyl) and dissociates above a certain threshold, which leads to tolerance and increased MOP phosphorylation, PKA- or PKC-mediated (Rodriguez-Munoz, Sanchez-Blazquez, Vicente-Sanchez, Berrocoso, & Garzon, 2012).

Hence, on the one hand, NMDAR channels have been found to be activated by endogenous opioid peptides (Tan-No et al., 2002) and on the other, to be inhibited by synthetic alkaloid opioids (Rodriguez-Munoz et al., 2012). This highlights that they may contribute to nociception as well as the onset of a tolerant profile in a ligand-dependent manner.

Importantly, a family of ion channels, the α9/α10-containing nicotinic acetylcholine receptors, were shown to be inhibited by opioid peptides in *Xenopus* oocytes (Lioudyno et al., 2002). It is therefore tempting to speculate that other ion channels are equally modulated by endogenous and/or exogenous opioids, independently of classical opioid receptors, however there are still few reports assessing such interaction.

2.5. Additional atypical receptors

Other proteins linked with opioid-like effects such as psychotomimesis or analgesia were initially classified as opioid receptors (Yaksh, 1984). This was the case for the sigma receptor, an endoplasmic-reticulum-resident transmembrane protein, which binds several synthetic opioid drugs but no endogenous opioid peptides (Hayashi & Su, 2005). This receptor was eventually removed from the classical OR family due to its unrelated structure and function (Cox et al., 2015).

Another putative opioid receptor was the epsilon receptor, introduced as a response to unattributed effects of endogenous opioid peptide β-endorphin in rat ileum and mouse vas deferens (Schulz, Wuster, & Herz, 1981). While some reports suggested that antinociception and release of Met-enkephalin were not mediated by classical ORs (Narita & Tseng, 1998), a triple MOP/KOP/DOP KO abolished these effects suggesting that the ε-receptor is either a splice variant or a heteromer of two or more ORs (Contet, Matifas, & Kieffer, 2004).

Interestingly, the opioid growth factor receptor (OGFr) or ζ-opioid receptor, which binds exclusively OGF (Met-enkephalin), is sensitized by naloxone (Zagon, Goodman, & McLaughlin, 1989). It is a non-GPCR receptor localized on the outer nuclear envelope, which is directed to the nucleus upon OGF binding, eventually preventing cell division. It is mainly expressed in the heart and liver, and to a lesser degree in the brain and pancreas. OGFr is not classified as a classical OR due to its uncharacteristic structure and its non-opioid related functions, mainly tissue and cell proliferation (Tanaka, Kondo, Hamamura, & Togari, 2019; Zagon et al., 1989).

3. Atypical opioid receptors - therapeutic opportunities

While research on opioid-related disorders, among them (chronic) pain and depression, has made steady progress, little improvement has been made in the development of new opioid receptor-targeting drugs with higher benefit/risk ratio. The insight on biased signaling offered a potential opportunity to fine-tune opioid action by dissociating the analgesic and adverse effects of opioid treatment. Recently, this led to the introduction of the G protein-biased FDA-approved drug TRV130 (oliceridine) (Mullard, 2020). However, despite tremendous efforts to find additional ligands with a similar pharmacological profile and to further characterize the extent of their functional selectivity in

relevant models, evidence for clinical benefit of such molecules remains frail and has been challenged repeatedly (Bachmutsky et al., 2021; Benredjem et al., 2019; Gillis et al., 2020; L. He et al., 2021; Kliewer et al., 2020).

Overall, the development of improved ligands of the four classical opioid receptors has shown limited success (Altarifi et al., 2017; Gillis et al., 2020; Hill et al., 2018). Therefore, it may be a potential alternative to target other opioid peptide-binding receptors, which may not be associated with the same impasses. In this context, the existent data on their biology and pharmacological modulation of both their opioid- and non-opioid-related functions represent a valuable asset, especially since some of their specific modulators have already been evaluated in the clinic.

3.1. Modulation of atypical opioid receptors by natural and synthetic opioids

Several prototypical opioid drugs can also directly modulate atypical ORs, and while in some instances these interactions may provide a therapeutic advantage, for others they may have negative effects.

For instance, a study looking at perioperative procedures and anesthesia pinpointed the interaction between natural (morphine) and synthetic (fentanyl) opioids with MrgX2. Such activation might be the leading cause of the characteristic IgE-independent mast cell degranulation observed in a number of patients treated with these analgesics (Navines-Ferrer et al., 2018).

NMDA receptors have also been shown to be modulated by chronic morphine administration (Feehan & Zadina, 2019). Moreover, a group of higher-efficacy MOP agonists seem to act as partial or weak NMDAR antagonists (Ebert, Thorkildsen, Andersen, Christrup, & Hjeds, 1998). This dual profile may be responsible for the decrease in adverse events they display. A relevant example is methadone, an opioid analgesic often used in opioid maintenance therapy, which in combination with morphine, leads to significantly lower morphine tolerance and dependence in preclinical models (He & Whistler, 2005; Sotgiu, Valente, Storchi, Caramenti, & Biella, 2009). Although this may be explained by the long half-life of methadone, it may also stem from its intrinsic antagonistic activity towards NMDA receptors, which prevents their upregulation and subsequent onset of tolerance and hyperalgesia.

On the other hand, ACKR3 was described to be unresponsive to a number of prototypical opioid drugs used in the clinic, including morphine, fentanyl, naloxone or methadone or the high-specificity ligands D-Ala^2,D-Leu^5-Enkephalin (DADLE) or [D-Ala^2, *N*-MePhe^4, Gly-ol]-enkephalin (DAMGO), potent agonists for DOP and MOP respectively (Meyrath et al., 2020). So far, there are no reports on the interaction of bradykinin receptors or MrgX1 with natural or synthetic opioids.

3.2. Direct pharmacological modulation of atypical opioid receptor signaling

Several studies have already assessed the effects of modulating the aforementioned receptors with their canonical agonists and antagonists in relation to opioid-related functions.

3.2.1. Mas-Related Receptors GPCR member X (MRGX)

Only a few molecules targeting MgrX1 have been described, including three different antagonist scaffolds (Bayrakdarian, Butterworth, Hu, Santhakumar, & Tomaszewski, 2011; Kunapuli et al., 2006; Schmidt, Butterworth, O'Donnell, Santhakumar, & Tomaszewski, 2009) and so far, no selective agonist is available for this receptor. Interestingly, it was shown that the positive allosteric modulator of MrgX1, ML382, in the presence of the endogenous ligand BAM8-22, and to a lesser extent alone, attenuates persistent nociception without significant adverse effects in humanized mice which underwent chronic constriction injury (Li et al., 2017). The same study also showed that BAM8-22, inactive at the MOP, is upregulated following spinal injury, which further legitimates the interest in its possible interaction with MrgX1 in chronic non-cancer pain, a condition hardly treatable with traditional MOP agonists due to the inevitable onset of tolerance.

Although the modulation of the receptor MrgX2, reported to be activated by dynorphin-related peptides, might influence opioid-related disorders, preclinical evidence has not yet been put forward, mainly due to the lack of potent and selective modulators. However, a highly specific agonist, ZINC-3573 was recently designed *in silico* and confirmed *in vitro* (Lansu et al., 2017), opening up the possibility to determine this receptor's role in pain modulation.

3.2.2. Bradykinin Receptors (BRs)

Bradykinin receptors also make for credible targets against pain, especially neuropathic pain. In such conditions, dynorphin A is upregulated and no longer mediates antinociception (Podvin, Yaksh, & Hook, 2016). One of the suggested pathways for this paradox is through BRs. A study a rat model of neuropathic pain together with naïve animals, found that the cleaved fragment dynorphin $A_{2\text{-}13}$, inactive on classical receptors, facilitates nociception transmission only in neuropathic animals, and that a B_2 antagonist counteracts this effect only in these animals (Bannister et al., 2014). This was supported by a cluster of other studies which found that B_2 antagonists such as HOE-140 reversed nerve injury-induced nociception, and that this effect was only observed in animals with upregulated spinal dynorphin but not bradykinin (Lai et al., 2006; Luo et al., 2008). This suggests that dynorphin is the main mediator of BR activation and subsequent hyperalgesia in this context. Hence, chronic nociception alters the physiology of the spinal environment and the main actors of noxious transmission may change accordingly, here relieving classical opioid receptors and giving way to alternative receptors such as BRs.

Therefore, targeting BRs may offer an opportunity to improve chronic pain management in a non-addictive approach which does not rely on classical ORs. In contrast to MrgX1 and 2, there are many available peptides and small molecules which modulate BRs, including the FDA-approved drug Icatibant, used against hereditary angioedema in adults, and Fasitibant, currently in Phase II clinical trials, which both inhibit B_2. FOV2304, also known as Safotibant is the only B_1 antagonist to reach clinical trials but, similarly to Icantibant and Fasitibant, it has not been evaluated in chronic pain conditions. An exhaustive list of B_1 antagonists and patents in the context of pain has been reviewed elsewhere (Bozo, Eles, & Keseru, 2012).

3.2.3. N-Methyl-D-aspartic acid receptors (NMDARs)

NMDARs have been repeatedly invoked in the discussion of pain management as they are involved in an array of opioid-related responses, including analgesia and the onset of tolerance.

In an early study, the analgesic efficacy of the co-administration of the NMDAR antagonist ketamine and the MOP agonist morphine was evaluated in postoperative pain control in patients undergoing joint replacement. It was shown that while ketamine alone did not produce pain relief, it lead to a stronger analgesic effect when combined with morphine treatment (Wong, Liaw, Tung, Su, & Ho, 1996). This generated interest in NMDAR inhibition in the context of pain management and the same group later found that competitive (D-AP5) or non-competitive (MK-801) NMDAR antagonists also potentiate the effect of morphine in rats and prevent the onset of tolerance (Wong, Cherng, Luk, Ho, & Tung, 1996). There are many commercially available NMDAR antagonists, which display different affinities for the receptor, correlating both with the extent of morphine-induced analgesia and the severity of side effects. Methadone, memantine or dextromethorphan all show weaker affinity for NMDARs than ketamine, which has shown the strongest morphine-sparing effect and reduced dependence (Kollender et al., 2008; Wong, Liaw, et al., 1996). However, it is associated with substantial dose-limiting effects such as dizziness, sedation, dissociation (Sang, 2000). An extensive review on NMDAR antagonism is available (Lipton, 2004).

Additionally, the interest in NMDARs as targets for pain management shed light on the downstream effects of opioid agonists beyond their typical OR-modulating actions. Indeed, some agonists have a differential impact on atypical OR activation as well, which may influence both analgesia and the onset of side effects. For instance, it was recently shown that the endomorphin analog MEL-0614 displays higher antinociception and less tolerance than morphine in an animal model of neuropathic pain (Ma et al., 2020). This added value was accredited to the lack of NMDAR activation upon sustained MEL-0614 administration, while chronic morphine treatment typically induces an NMDAR response. Importantly, the hyperalgesia and tolerance developed with morphine were blocked by dynorphin A-specific antibodies but not by naltrexone, suggesting these effects are not mediated by classical OR. Instead, they may stem from the interaction with NMDARs, considering the affinity of dynorphin A for the receptor's polyamine sites that drive excitatory signals (Caudle & Dubner, 1998).

Interestingly, a cluster of studies has shown that the hyperalgesic phenotype associated with continuous morphine treatment is both sex- and hormone-dependent in mice, and that male and ovariectomized female hyperalgesia could be reversed by NMDAR antagonists, while this was not the case for gonadally intact females (Juni et al., 2010; Juni, Klein, Kowalczyk, Ragnauth, & Kest, 2008; Waxman et al., 2010).

3.3. Indirect modulation of classical ORs by atypical OR

3.3.1. Inhibition of scavenging mediated by the atypical opioid receptor ACKR3

The approach of regulating opioid-dependent effects by increasing the availability of active opioid peptides has already been investigated with the development of enkephalinase inhibitors, which restore the 'natural' antinociception of endogenous enkephalins. Such inhibitors have shown antinociceptive properties without the advent of side effects (Noble, Turcaud, Fournie-Zaluski, & Roques, 1992; Szymaszkiewicz, Storr, Fichna, & Zielinska, 2019). This supports the potential of targeting ACKR3, which was recently discovered to selectively scavenge endogenous enkephalin, dynorphin and nociceptin peptides, as another avenue for opioid fine-tuning. Indeed, the affinity of ACKR3 for endogenous MOP agonists like proenkephalin-derived peptides suggests that blocking its scavenging function may enhance the concentration of endogenous BAM22 or adrenorphin peptides, making them more available to classical ORs. This concept has been supported by a couple of studies already. Indeed, targeting ACKR3 with the highly specific small-molecule compound CCX771 was described to have, synergistically with ACTH, an anxiolytic-like effect on behavior in mice (Ikeda et al., 2013). Moreover, the effect of adrenorphin-derived small peptide LIH383, which blocks ACKR3 scavenging function, was recently addressed in an *ex vivo* rat locus coeruleus model where it potentiates the effect of endogenous opioids (Meyrath et al., 2020).

Besides CCX771, other analogues have been developed with various pharmacological profiles, including the partial agonist CCX777 (Gustavsson et al., 2017) or CCX733 (Hartmann et al., 2008). Together with VUF11207 and VUF11403, two small-molecule agonists designed on a similar scaffold (Wijtmans et al., 2012), these compounds are valuable tools for investigating ACKR3 biology and may lead to the development of novel opioid therapeutics. Recently, a diphenylacetamide analogue and ACT-1004-1239 were reported as the first antagonists of ACKR3 (Menhaji-Klotz et al., 2020; Richard-Bildstein et al., 2020). The latter was recently tested in human for safety, tolerability, pharmacokinetics, and pharmacodynamics in a multipurpose study using CXCL12 plasma concentration as target engagement biomarker and reinforced ACKR3 as a valuable drug target (Huynh et al., 2021). Interestingly, ACKR3 has recently been demonstrated to be the main GPCR target of conolidine (Szpakowska et al., 2021), a natural analgesic alkaloid found in the bark of the tropical flowering shrub *Tabernaemontana divaricate*, which is used in traditional Chinese medicine to treat fever and pain (Tarselli et al., 2011). This provides additional correlative evidence between ACKR3 and pain modulation. Systematic chemical modifications of conolidine resulted in a analogue compound, RTI-5152-12, with 15-fold improved potency towards ACKR3. Notably, conolidine and RTI-5152-12 function similarly to LIH383 and conolidine's analgesic activity was proposed to rely on the inhibition of the scavenging functions of ACKR3 increasing the availability of analgesia-inducing endogenous opioid peptides for the classical ORs. Considering the plausible non-signaling function of ACKR3, both antagonists blocking the receptor or agonists competing with the uptake and scavenging of the opioid peptides could be valuable. The current state of ACKR3 pharmacological modulation has been recently reviewed extensively (Adlere et al., 2019; Lounsbury, 2020).

3.3.2. Modulation of classical ORs by heterodimerization with atypical OR

Classical ORs were shown to form heterodimers with several GPCRs, including the atypical ORs. These allosteric interactions often result in modified pharmacology (as defined by the IUPHAR nomenclature of multimeric G protein-coupled receptors (Pin et al., 2007)) and can lead to alternative downstream effects for instance through coupling to different G proteins. Heterologous desensitization, or phosphorylation and G protein uncoupling of a primary receptor in the absence of its ligand by kinases activated by the stimulation of a secondary receptor has been shown for several receptors, including MOP and the neurokinin 1 receptor (NK1R) (Bowman et al., 2015) and may occur through heterodimerization of classical ORs with atypical ORs.

Evidence pointing to heterodimerization of DOP with MrgX1 were first provided by BRET titration experiments in HEK293 cells (Breit, Gagnidze, Devi, Lagace, & Bouvier, 2006). Both receptors are activated by BAM22, which typically leads to inhibition of adenylyl cyclase or activation of phospholipase C for DOP and MrgX1, respectively. It was shown, however, that upon transient co-expression of the two receptors in HEK293 cells, BAM22 potentiates MrgX1 signaling but no longer promotes DOR-mediated inhibition of cAMP production (Breit et al., 2006). Similar observations were made in cultured neurons from DRG. While treatment with the DOP ligand Leu-Enkephalin predictably reduced cAMP accumulation, co-treatment with BAM8-22 inhibited this effect, supposedly owing to MrgX1 acting as an agonist-dependent dominant negative receptor towards DOR (Breit et al., 2006). More recently, MrgC, the mouse homolog of MrgX1, was shown to oligomerize with MOP and to promote fast recycling of the complex to the cell surface, potentiating morphine antinociception *in vivo*. The ability of MOP and MrgX1 to heterodimerize was shown in HEK293 cells, however further characterization of the potential functional cellular outcomes of this interaction is warranted (He et al., 2018).

In vitro, B_2 was shown to form heterodimers with KOP, leading to a shift in G protein coupling specificity of the latter. Indeed, B_2–KOP heterodimers showed enhanced interactions with $G_{s,}$ compared to each receptor alone, which was concomitant with a decrease in KOR–$G_{i/o}$ interaction and an increase in cAMP levels following dynorphin $A_{1\text{-}13}$ stimulation (Ji et al., 2017). Interestingly, B_2 ligand bradykinin did not induce cAMP signaling through the B_2–KOP heterodimer, as was observed with B_2 alone, suggesting a change in ligand-receptor interaction upon receptor heterodimerization.

NMDA receptors have also been found to colocalize with opioid receptors in individual neurons of the CNS, including the PAG region (Commons et al., 1999; Rodriguez-Munoz et al., 2012). The interaction between NMDARs and MOP has been further elucidated, and shown to be modulated by exogenous MOP agonists (Rodriguez-Munoz et al., 2012). Indeed, morphine disrupts the MOR–NMDAR complex and potentiates NMDAR-mediated signaling, contributing to morphine tolerance. NMDAR agonists equally separate the complex and diminishe the antinociceptive potential of morphine. Overall, this dimer formation shows a positive allosteric modulation on the MOP and a negative allosteric modulation on the NMDAR ion channel, providing corroborating evidence of functional heterodimerization.

Although it remains to be investigated, ACKR3 may be able to heterodimerize with classical opioid receptors and modulate their signaling properties or sensitization, in analogy to its regulatory function of CXCR4 within the chemokine system (Meyrath et al., 2020).

Therefore, targeting heterodimers between classical and atypical ORs may allow alternative means for receptor modulation or even rerouting of their downstream effects and thus should be considered as another strategy to modulate the opioid system.

4. Discussion and perspectives

4.1. Atypical opioid receptors - dual-activity receptors relevant in the opioid system

As stated by the IUPHAR in 2015, the nomenclature for opioid receptors has been a moving subject. To this day, the family of classical ORs is restricted to the μ, δ, κ and nociceptin receptors, or MOP, DOR, KOP and NOP. With the exception of the latter, they are bound by their sensitivity to naloxone/naltrexone. However, NOP's high sequence homology to other classical ORs qualifies it as such. On the other hand, atypical ORs are not inhibited by unselective antagonists such as naloxone and are phylogenetically distinct from classical ORs. Furthermore, atypical ORs are associated with another primary function, which does not relate directly to opioidergic behaviors—*e.g.* BRs with inflammation, NMDARs with excitatory synaptic signals or ACKR3 with maintenance of chemokine homeostasis. However, there is leading evidence both *in vitro* and *in vivo* that atypical ORs bind endogenous opioid peptides selectively and, more importantly, directly impact opioid-related phenotypes, including antinociception and anxiety, which supports their roles as physiological ORs rather than mediators of off-target effects stemming from their ligand binding promiscuity (Table 1).

4.2. Diversity and unity of atypical ORs and similarities with classical ORs

As covered in this review, atypical ORs form a heterogeneous group of receptors, including class A GPCRs but also ligand-gated ion channels. The primary—non-opioid-related—functions of these receptors range from homeostasis and chemotaxis maintenance to vasodilatation, anaphylactic response and excitatory neurotransmission (Fig. 1).

ACKR3 and MrgX2 are fairly promiscuous towards the opioid peptide families. ACKR3 shows high affinity towards several peptides from the enkephalin and dynorphin family, in line with its function as a broad-spectrum opioid scavenger (Meyrath et al., 2020). This is also the case for MrgX2, which binds to BAM8-22 but also several prodynorphin-derived fragments (Lansu et al., 2017). In contrast, NMDARs, BRs and MrgX1 only bind one or a few endogenous opioid peptides. MrgX1 binds the proenkephalin-derived peptide BAM22 (and processed BAM8-22) while BRs and NMDARs bind dynorphin A and truncated variants, as well as big dynorphin for the latter (Figs. 1 and 2).

One may draw a parallel between classical and atypical ORs regarding their ligand binding spectra: MrgX1 shows an affinity for enkephalins, reminiscent of MOP, BRs and NMDARs exhibit KOP-like selectivity patterns towards dynorphins, while MrgX2 binds enkephalin and dynorphin derivatives equally (Fig. 1). Interestingly, an adrenorphin SAR analysis showed that ACKR3 can morph from a MOP, KOP to NOP-like activation profile in response to different adrenorphin mutants. Hence, similarly to classical OR, atypical ORs have specific affinity profiles for the different families of endogenous opioid peptides. Noteworthy, all atypical ORs respond to endogenous full-length opioid peptides but also to truncated enkephalin and dynorphin variants, such as BAM8-22, dynorphin 2-13 and dynorphin 2-17, which were shown to have a physiological effect but are inactive on classical ORs (Gac, Butterick, Duffy, Teske, & Perez-Leighton, 2016; Walker, Moises, Coy, Baldrighi, & Akil, 1982) (Fig. 2C).

With the exception of NMDARs, atypical ORs identified to date are peptide-binding class A GPCRs with affinities for endogenous opioid peptides ranging from nanomolar to low micromolar. In contrast to classical ORs that exclusively signal through $G_{i/o}$ proteins, atypical ORs modulate opioid-related phenotypes through several modes of action and signaling routes: the MrgX receptors along with the BRs are signaling GPCRs, inducing $G_{i/o}$ but also $G_{q/11}$ responses. In contrast, the evidence for ACKR3 signaling is still contentious and its main modus operandi is proposed to be through scavenging of endogenous peptides. NMDARs, as ion-channels, rely on yet an alternate pathway following dynorphin A or big dynorphin binding, although the exact mechanisms responsible for the induction of pain or anxiolytic-like behavior respectively have not been elucidated as of yet.

4.3. Open questions and challenges

While targeting atypical ORs with specific modulators may appear as a promising new therapeutic avenue, there is only scarce knowledge about some of these receptors' implication in opioid-related disorders and often limited pharmacological tools are available. Although some atypical ORs show nanomolar affinities towards opioid peptides (e.g. ACKR3 or MrgX2), others may have affinities that are several orders of magnitude lower than classical ORs. However, accurate comparative studies on the modulation of classical and atypical ORs by opioid peptides are still missing. Moreover, as atypical ORs are all involved in diverse pathways, the potential side effects associated with their inhibition or potentiation should be considered, based on both their primary functions and opioid-related behaviors. Additionally, the possible activity on atypical ORs of other bioactive peptides generated during the processing of opioid precursors (*e.g.* ACTH, MSH from POMC and OFQ2 from PNOC) and inactive on classical opioid receptors should be further characterized, as was initiated for MrgX1 (Lembo et al., 2002).

Drugs targeting atypical ORs have shown efficacy in different *in vitro* and *in vivo* models and opened up new and unanticipated avenues for drug development, relying on molecules with completely different modes of action and pharmacology than classical opioids. Importantly, the development of synthetic opioids may also benefit from considering the interplay of these drugs with atypical ORs. Indeed, opioid drugs with weak activity on atypical OR, such as NMDAR antagonism, could also yield a higher benefit/risk ratio, among which methadone and ketobemidone are pertinent examples.

Altogether, atypical ORs appear as promising emerging pharmacological targets, but significant efforts must be undertaken to clarify and determine the extent of their role in pain modulation, anxiety and depression. This should include their modulation, alone or in combination with classical OR-targeting drugs, as well as the potential side-effects, both in preclinical models and human studies.

Most atypical ORs have been evaluated in the context of pain, anxiety, memory or other opioid-related phenotypes to some extent and there is strong preclinical evidence for their relevance as an alternative means to modulate the opioid machinery. This should be extended to human studies, as some of the atypical OR modulators have only been clinically evaluated in the context of their 'primary' function, while their FDA-approved drugs could prove highly beneficial to opioid-related disorders. Importantly, additional studies must be conducted for ACKR3 and MrgX2 to validate and characterize both their role in nociception and the potential side effects of their modulation.

It should be noted that there are probably other receptors or ion channels binding opioid peptides, possibly among orphan receptors (Fricker & Devi, 2018), and their discovery remains challenging given their as yet unknown signaling mechanisms. While the opioid receptor family is currently restricted to four receptors, the proposition of a subfamily of atypical ORs allows an extended overview of the possible actors involved in opioid-related disorders. Exploiting the breadth of variety within this family, such as the ligand binding patterns, expression profiles and modes of action, while considering the dynamic context of such disorders may offer new therapeutic opportunities in

direct or combined therapies to hopefully broaden and improve the opioid-associated pharmacopoeia.

Conflict of interest statement

The authors declare no conflict of interest.

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References

Adlere, I., Caspar, B., Arimont, M., Dekkers, S., Visser, K., Stuijt, J., de Graaf, C., Stocks, M., Kellam, B., Briddon, S., Wijtmans, M., de Esch, I., Hill, S., & Leurs, R. (2019). Modulators of CXCR4 and CXCR7/ACKR3 function. *Molecular Pharmacology 96*(6), 737–752.

Al-Hasani, R., & Bruchas, M. R. (2011). Molecular mechanisms of opioid receptor-dependent signaling and behavior. *Anesthesiology 115*(6), 1363–1381.

Altarifi, A. A., David, B., Muchhala, K. H., Blough, B. E., Akbarali, H., & Negus, S. S. (2017). Effects of acute and repeated treatment with the biased mu opioid receptor agonist TRV130 (oliceridine) on measures of antinociception, gastrointestinal function, and abuse liability in rodents. *Journal of Psychopharmacology 31*(6), 730–739.

Alvarez-Curto, E., Inoue, A., Jenkins, L., Raihan, S. Z., Prihandoko, R., Tobin, A. B., & Milligan, G. (2016). Targeted elimination of G proteins and arrestins defines their specific contributions to both intensity and duration of G protein-coupled receptor signaling. *The Journal of Biological Chemistry 291*(53), 27147–27159.

Bachelerie, F., Ben-Baruch, A., Burkhardt, A. M., Combadiere, C., Farber, J. M., Graham, G. J., ... Zlotnik, A. (2014). International Union of Basic and Clinical Pharmacology. [corrected]. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. *Pharmacological Reviews 66*(1), 1–79.

Bachmutsky, I., Wei, X. P., Durand, A., & Yackle, K. (2021). Beta-arrestin 2 germline knockout does not attenuate opioid respiratory depression. *Elife 10*.

Bader, M., Alenina, N., Andrade-Navarro, M. A., & Santos, R. A. (2014). MAS and its related G protein-coupled receptors, Mrgprs. *Pharmacological Reviews 66*(4), 1080–1105.

Bailey, C. P., & Connor, M. (2005). Opioids: Cellular mechanisms of tolerance and physical dependence. *Current Opinion in Pharmacology 5*(1), 60–68.

Balabanian, K., Lagane, B., Infantino, S., Chow, K. Y., Harriague, J., Moepps, B., ... Bachelerie, F. (2005). The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. *The Journal of Biological Chemistry 280*(42), 35760–35766.

Bannister, K., Lee, Y. S., Goncalves, L., Porreca, F., Lai, J., & Dickenson, A. H. (2014). Neuropathic plasticity in the opioid and non-opioid actions of dynorphin A fragments and their interactions with bradykinin B2 receptors on neuronal activity in the rat spinal cord. *Neuropharmacology 85*, 375–383.

Bayrakdarian, M., Butterworth, J., Hu, Y. J., Santhakumar, V., & Tomaszewski, M. J. (2011). Development of 2,4-diaminopyrimidine derivatives as novel SNSR4 antagonists. *Bioorganic & Medicinal Chemistry Letters 21*(7), 2102–2105.

Benredjem, B., Gallion, J., Pelletier, D., Dallaire, P., Charbonneau, J., Cawkill, D., Nagi, K., Gosink, M., Lukasheva, V., Jenkinson, S., Ren, Y., Somps, C., Murat, B., Van Der Westhuizen, E., Le Gouill, C., Lichtarge, O., Schmidt, A., Bouvier, M., & Pineyro, G. (2019). Exploring use of unsupervised clustering to associate signaling profiles of GPCR ligands to clinical response. *Nature Communications 10*(1), 4075.

Bertram, C. M., Baltic, S., Misso, N. L., Bhoola, K. D., Foster, P. S., Thompson, P. J., & Fogel-Petrovic, M. (2007). Expression of kinin B1 and B2 receptors in immature, monocyte-derived dendritic cells and bradykinin-mediated increase in intracellular Ca2+ and cell migration. *Journal of Leukocyte Biology 81*(6), 1445–1454.

Bohn, L. M., Lefkowitz, R. J., Gainetdinov, R. R., Peppel, K., Caron, M. G., & Lin, F. T. (1999). Enhanced morphine analgesia in mice lacking beta-arrestin 2. *Science 286*(5449), 2495–2498.

Bowman, S. L., Soohoo, A. L., Shiwarski, D. J., Schulz, S., Pradhan, A. A., & Puthenveedu, M. A. (2015). Cell-autonomous regulation of Mu-opioid receptor recycling by substance P. *Cell Reports 10*(11), 1925–1936.

Bozo, E., Eles, J., & Keseru, G. M. (2012). Bradykinin B1 receptor antagonists: a patent update 2009–2012. *Expert Opinion on Therapeutic Patents 22*(12), 1443–1452.

Breit, A., Gagnidze, K., Devi, L. A., Lagace, M., & Bouvier, M. (2006). Simultaneous activation of the delta opioid receptor (deltaOR)/sensory neuron-specific receptor-4 (SNSR-4) hetero-oligomer by the mixed bivalent agonist bovine adrenal medulla peptide 22 activates SNSR-4 but inhibits deltaOR signaling. *Molecular Pharmacology 70*(2), 686–696.

Bruchas, M. R., Land, B. B., Aita, M., Xu, M., Barot, S. K., Li, S., & Chavkin, C. (2007). Stress-induced p38 mitogen-activated protein kinase activation mediates kappa-opioid-dependent dysphoria. *The Journal of Neuroscience 27*(43), 11614–11623.

Bruchas, M. R., Macey, T. A., Lowe, J. D., & Chavkin, C. (2006). Kappa opioid receptor activation of p38 MAPK is GRK3- and arrestin-dependent in neurons and astrocytes. *The Journal of Biological Chemistry 281*(26), 18081–18089.

Buntin-Mushock, C., Phillip, L., Moriyama, K., & Palmer, P. P. (2005). Age-dependent opioid escalation in chronic pain patients. *Anesthesia and Analgesia 100*(6), 1740–1745.

Burford, N. T., Clark, M. J., Wehrman, T. S., Gerritz, S. W., Banks, M., O'Connell, J., ... Alt, A. (2013). Discovery of positive allosteric modulators and silent allosteric modulators of the mu-opioid receptor. *Proceedings of the National Academy of Sciences of the United States of America 110*(26), 10830–10835.

Burford, N. T., Traynor, J. R., & Alt, A. (2015). Positive allosteric modulators of the mu-opioid receptor: A novel approach for future pain medications. *British Journal of Pharmacology 172*(2), 277–286.

Burgess, G. M., Mullaney, I., McNeill, M., Dunn, P. M., & Rang, H. P. (1989). Second messengers involved in the mechanism of action of bradykinin in sensory neurons in culture. *The Journal of Neuroscience 9*(9), 3314–3325.

Burns, J. M., Summers, B. C., Wang, Y., Melikian, A., Berahovich, R., Miao, Z., ... Schall, T. J. (2006). A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *The Journal of Experimental Medicine 203*(9), 2201–2213.

Burstein, E. S., Ott, T. R., Feddock, M., Ma, J. N., Fuhs, S., Wong, S., ... Nash, N. R. (2006). Characterization of the Mas-related gene family: Structural and functional conservation of human and rhesus MrgX receptors. *British Journal of Pharmacology 147*(1), 73–82.

Caudle, R. M., & Dubner, R. (1998). Ifenprodil blocks the excitatory effects of the opioid peptide dynorphin 1-17 on NMDA receptor-mediated currents in the CA3 region of the guinea pig hippocampus. *Neuropeptides 32*(1), 87–95.

CDC, & N. C. f. H. S (2021). Wide-ranging online data for epidemiologic research (WONDER). available at https://www.cdc.gov/drugoverdose/data/ Atlanta, GA.

Chavkin, C., Schattauer, S. S., & Levin, J. R. (2014). Arrestin-mediated activation of p38 MAPK: Molecular mechanisms and behavioral consequences. *Handbook of Experimental Pharmacology 219*, 281–292.

Chen, L., Gu, Y., & Huang, L. Y. (1995). The opioid peptide dynorphin directly blocks NMDA receptor channels in the rat. *The Journal of Physiology 482*(Pt 3), 575–581.

Chen, L., & Huang, L. Y. (1998). Dynorphin block of N-methyl-D-aspartate channels increases with the peptide length. *The Journal of Pharmacology and Experimental Therapeutics 284*(3), 826–831.

Chen, Y., Mestek, A., Liu, J., Hurley, J. A., & Yu, L. (1993). Molecular cloning and functional expression of a mu-opioid receptor from rat brain. *Molecular Pharmacology 44*(1), 8–12.

Commons, K. G., van Bockstaele, E. J., & Pfaff, D. W. (1999). Frequent colocalization of mu opioid and NMDA-type glutamate receptors at postsynaptic sites in periaqueductal gray neurons. *Journal of Comparative Neurology 408*(4), 549–559.

Connor, M., & Christie, M. D. (1999). Opioid receptor signalling mechanisms. *Clinical and Experimental Pharmacology & Physiology 26*(7), 493–499.

Contet, C., Matifas, A., & Kieffer, B. L. (2004). No evidence for G-protein-coupled epsilon receptor in the brain of triple opioid receptor knockout mouse. *European Journal of Pharmacology 492*(2–3), 131–136.

Cox, B. M., Christie, M. J., Devi, L., Toll, L., & Traynor, J. R. (2015). Challenges for opioid receptor nomenclature: IUPHAR Review 9. *British Journal of Pharmacology 172*(2), 317–323.

Davenport, A. P., Alexander, S. P., Sharman, J. L., Pawson, A. J., Benson, H. E., Monaghan, A. E., ... Harmar, A. J. (2013). International Union of Basic and Clinical Pharmacology. LXXXVIII. G protein-coupled receptor list: recommendations for new pairings with cognate ligands. *Pharmacological Reviews 65*(3), 967–986.

DeWire, S. M., Yamashita, D. S., Rominger, D. H., Liu, G., Cowan, C. L., Graczyk, T. M., ... Violin, J. D. (2013). A G protein-biased ligand at the mu-opioid receptor is potently analgesic with reduced gastrointestinal and respiratory dysfunction compared with morphine. *The Journal of Pharmacology and Experimental Therapeutics 344*(3), 708–717.

Dhamrait, S. S., Payne, J. R., Li, P., Jones, A., Toor, I. S., Cooper, J. A., ... Montgomery, H. E. (2003). Variation in bradykinin receptor genes increases the cardiovascular risk associated with hypertension. *European Heart Journal 24*(18), 1672–1680.

Dinges, H. C., Otto, S., Stay, D. K., Baumlein, S., Waldmann, S., Kranke, P., ... Eberhart, L. H. (2019). Side effect rates of opioids in equianalgesic doses via intravenous patient-controlled analgesia: A systematic review and network meta-analysis. *Anesthesia and Analgesia 129*(4), 1153–1162.

Dowell, D., Haegerich, T. M., & Chou, R. (2016). CDC guideline for prescribing opioids for chronic pain - United States, 2016. *MMWR - Recommendations and Reports 65*(1), 1–49.

Dumas, E. O., & Pollack, G. M. (2008). Opioid tolerance development: a pharmacokinetic/pharmacodynamic perspective. *The AAPS Journal 10*(4), 537–551.

Ebert, B., Thorkildsen, C., Andersen, S., Christrup, L. L., & Hjeds, H. (1998). Opioid analgesics as noncompetitive N-methyl-D-aspartate (NMDA) antagonists. *Biochemical Pharmacology 56*(5), 553–559.

Ehrenfeld, P., Millan, C., Matus, C. E., Figueroa, J. E., Burgos, R. A., Nualart, F., ... Figueroa, C. D. (2006). Activation of kinin B1 receptors induces chemotaxis of human neutrophils. *Journal of Leukocyte Biology 80*(1), 117–124.

Ehrlich, A. T., Semache, M., Gross, F., Da Fonte, D. F., Runtz, L., Colley, C., ... Kieffer, B. L. (2019). Biased signaling of the Mu opioid receptor revealed in native neurons. *iScience 14*, 47–57.

Evans, C. J., Keith, D. E., Jr., Morrison, H., Magendzo, K., & Edwards, R. H. (1992). Cloning of a delta opioid receptor by functional expression. *Science 258*(5090), 1952–1955.

Feehan, A. K., & Zadina, J. E. (2019). Morphine immunomodulation prolongs inflammatory and postoperative pain while the novel analgesic ZH853 accelerates recovery and protects against latent sensitization. *Journal of Neuroinflammation 16*(1), 100.

Fichna, J., Janecka, A., Costentin, J., & Do Rego, J. C. (2007). The endomorphin system and its evolving neurophysiological role. *Pharmacological Reviews 59*(1), 88–123.

Fricker, L. D., & Devi, L. A. (2018). Orphan neuropeptides and receptors: Novel therapeutic targets. *Pharmacology & Therapeutics 185*, 26–33.

Fumagalli, A., Heuninck, J., Pizzoccaro, A., Moutin, E., Koenen, J., Seveno, M., ... Marin, P. (2020). The atypical chemokine receptor 3 interacts with Connexin 43 inhibiting astrocytic gap junctional intercellular communication. *Nature Communications 11*(1), 4855.

Gac, L., Butterick, T. A., Duffy, C. M., Teske, J. A., & Perez-Leighton, C. E. (2016). Role of the non-opioid dynorphin peptide des-Tyr-dynorphin (DYN-A(2-17)) in food intake and physical activity, and its interaction with orexin-A. *Peptides 76*, 14–18.

Gilbert, P. E., & Martin, W. R. (1976). The effects of morphine and nalorphine-like drugs in the nondependent, morphine-dependent and cyclazocine-dependent chronic spinal dog. *The Journal of Pharmacology and Experimental Therapeutics 198*(1), 66–82.

Gillis, A., Gondin, A. B., Kliewer, A., Sanchez, J., Lim, H. D., Alamein, C., ... Canals, M. (2020). Low intrinsic efficacy for G protein activation can explain the improved side effect profiles of new opioid agonists. *Science Signaling 13*(625).

Gomes, I., Sierra, S., Lueptow, L., Gupta, A., Gouty, S., Margolis, E. B., ... Devi, L. A. (2020). Biased signaling by endogenous opioid peptides. *Proceedings of the National Academy of Sciences of the United States of America 117*(21), 11820–11828.

Grundmann, M., Merten, N., Malfacini, D., Inoue, A., Preis, P., Simon, K., ... Kostenis, E. (2018). Lack of beta-arrestin signaling in the absence of active G proteins. *Nature Communications 9*(1), 341.

Gunther, T., Dasgupta, P., Mann, A., Miess, E., Kliewer, A., Fritzwanker, S., Steinborn, R., & Schulz, S. (2018). Targeting multiple opioid receptors - Improved analgesics with reduced side effects? *British Journal of Pharmacology 175*(14), 2857–2868.

Gustavsson, M., Wang, L., van Gils, N., Stephens, B. S., Zhang, P., Schall, T. J., ... Handel, T. M. (2017). Structural basis of ligand interaction with atypical chemokine receptor 3. *Nature Communications 8*, 14135.

Hackler, L., Zadina, J. E., Ge, L. J., & Kastin, A. J. (1997). Isolation of relatively large amounts of endomorphin-1 and endomorphin-2 from human brain cortex. *Peptides 18*(10), 1635–1639.

Hamilton, G. R., & Baskett, T. F. (2000). In the arms of Morpheus the development of morphine for postoperative pain relief. *Canadian Journal of Anaesthesia 47*(4), 367–374.

Hartmann, T. N., Grabovsky, V., Pasvolsky, R., Shulman, Z., Buss, E. C., Spiegel, A., ... Alon, R. (2008). A crosstalk between intracellular CXCR7 and CXCR4 involved in rapid CXCL12-triggered integrin activation but not in chemokine-triggered motility of human T lymphocytes and CD34+ cells. *Journal of Leukocyte Biology 84*(4), 1130–1140.

Hayashi, T., & Su, T. (2005). The sigma receptor: Evolution of the concept in neuropsychopharmacology. *Current Neuropharmacology 3*(4), 267–280.

He, L., Gooding, S. W., Lewis, E., Felth, L. C., Gaur, A., & Whistler, J. L. (2021). Pharmacological and genetic manipulations at the mu-opioid receptor reveal arrestin-3 engagement limits analgesic tolerance and does not exacerbate respiratory depression in mice. *Neuropsychopharmacology*. https://doi.org/10.1038/s41386-021-01054-x.

He, L., & Whistler, J. L. (2005). An opiate cocktail that reduces morphine tolerance and dependence. *Current Biology 15*(11), 1028–1033.

He, S. Q., Li, Z., Chu, Y. X., Han, L., Xu, Q., Li, M., ... Guan, Y. (2014). MrgC agonism at central terminals of primary sensory neurons inhibits neuropathic pain. *Pain 155*(3), 534–544.

He, S. Q., Xu, Q., Tiwari, V., Yang, F., Anderson, M., Chen, Z., ... Guan, Y. (2018). Oligomerization of MrgC11 and mu-opioid receptors in sensory neurons enhances morphine analgesia. *Science Signaling 11*(535).

Henderson, G., & McKnight, A. T. (1997). The orphan opioid receptor and its endogenous ligand—nociceptin/orphanin FQ. *Trends in Pharmacological Sciences 18*(8), 293–300.

Heuninck, J., Perpina Viciano, C., Isbilir, A., Caspar, B., Capoferri, D., Briddon, S. J., ... Hoffmann, C. (2019). Context-dependent signaling of CXC chemokine receptor 4 and atypical chemokine receptor 3. *Molecular Pharmacology 96*(6), 778–793.

Hider-Mlynarz, K., Cavalie, P., & Maison, P. (2018). Trends in analgesic consumption in France over the last 10 years and comparison of patterns across Europe. *British Journal of Clinical Pharmacology 84*(6), 1324–1334.

Hill, R., Disney, A., Conibear, A., Sutcliffe, K., Dewey, W., Husbands, S., Bailey, C., Kelly, E., & Henderson, G. (2018). The novel mu-opioid receptor agonist PZM21 depresses respiration and induces tolerance to antinociception. *British Journal of Pharmacology 175*(13), 2653–2661.

Hiramatsu, M., & Inoue, K. (2000). Des-tyrosine(1) dynorphin A-(2-13) improves carbon monoxide-induced impairment of learning and memory in mice. *Brain Research 859*(2), 303–310.

Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A., & Morris, H. R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature 258*(5536), 577–580.

Huynh, C., Henrich, A., Strasser, D. S., Boof, M. L., Al-Ibrahim, M., Meyer Zu Schwabedissen, H. E., ... Ufer, M. (2021). A multipurpose first-in-human study with the novel CXCR7 antagonist ACT-1004-1239 using CXCL12 plasma concentrations as target engagement biomarker. *Clinical Pharmacology and Therapeutics 109*(6), 1648–1659.

Ikeda, Y., Kumagai, H., Skach, A., Sato, M., & Yanagisawa, M. (2013). Modulation of circadian glucocorticoid oscillation via adrenal opioid-CXCR7 signaling alters emotional behavior. *Cell 155*(6), 1323–1336.

Janecka, A., Fichna, J., & Janecki, T. (2004). Opioid receptors and their ligands. *Current Topics in Medicinal Chemistry 4*(1), 1–17.

Ji, B., Liu, H., Zhang, R., Jiang, Y., Wang, C., Li, S., Chen, J., & Bai, B. (2017). Novel signaling of dynorphin at kappa-opioid receptor/bradykinin B2 receptor heterodimers. *Cellular Signalling 31*, 66–78.

Juni, A., Cai, M., Stankova, M., Waxman, A. R., Arout, C., Klein, G., ... Kest, B. (2010). Sex-specific mediation of opioid-induced hyperalgesia by the melanocortin-1 receptor. *Anesthesiology 112*(1), 181–188.

Juni, A., Klein, G., Kowalczyk, B., Ragnauth, A., & Kest, B. (2008). Sex differences in hyperalgesia during morphine infusion: Effect of gonadectomy and estrogen treatment. *Neuropharmacology 54*(8), 1264–1270.

Kamohara, M., Matsuo, A., Takasaki, J., Kohda, M., Matsumoto, M., Matsumoto, S., Soga, T., Hiyama, H., Kobori, M., & Katou, M. (2005). Identification of MrgX2 as a human G-protein-coupled receptor for proadrenomedullin N-terminal peptides. *Biochemical and Biophysical Research Communications 330*(4), 1146–1152.

Kandasamy, R., Hillhouse, T. M., Livingston, K. E., Kochan, K. E., Meurice, C., Eans, S. O., ... Traynor, J. R. (2021). Positive allosteric modulation of the mu-opioid receptor produces analgesia with reduced side effects. *Proceedings of the National Academy of Sciences of the United States of America 118*(16).

Kawaguchi, A., Sato, M., Kimura, M., Yamazaki, T., Yamamoto, H., Tazaki, M., Ichinohe, T., & Shibukawa, Y. (2015). Functional expression of bradykinin B1 and B2 receptors in neonatal rat trigeminal ganglion neurons. *Frontiers in Cellular Neuroscience 9*, 229.

Kliewer, A., Gillis, A., Hill, R., Schmiedel, F., Bailey, C., Kelly, E., ... Schulz, S. (2020). Morphine-induced respiratory depression is independent of beta-arrestin2 signalling. *British Journal of Pharmacology 177*(13), 2923–2931.

Kliewer, A., Schmiedel, F., Sianati, S., Bailey, A., Bateman, J. T., Levitt, E. S., ... Schulz, S. (2019). Phosphorylation-deficient G-protein-biased mu-opioid receptors improve analgesia and diminish tolerance but worsen opioid side effects. *Nature Communications 10*(1), 367.

Koenen, J., Bachelerie, F., Balabanian, K., Schlecht-Louf, G., & Gallego, C. (2019). Atypical chemokine receptor 3 (ACKR3): A comprehensive overview of its expression and potential roles in the immune system. *Molecular Pharmacology 96*(6), 809–818.

Kollender, Y., Bickels, J., Stocki, D., Maruoani, N., Chazan, S., Nirkin, A., ... Weinbroum, A. A. (2008). Subanaesthetic ketamine spares postoperative morphine and controls pain better than standard morphine does alone in orthopaedic-oncological patients. *European Journal of Cancer 44*(7), 954–962.

Kunapuli, P., Lee, S., Zheng, W., Alberts, M., Kornienko, O., Mull, R., ... Strulovici, B. (2006). Identification of small molecule antagonists of the human mas-related gene-X1 receptor. *Analytical Biochemistry 351*(1), 50–61.

Kuzmin, A., Madjid, N., Terenius, L., Ogren, S. O., & Bakalkin, G. (2006). Big dynorphin, a prodynorphin-derived peptide produces NMDA receptor-mediated effects on memory, anxiolytic-like and locomotor behavior in mice. *Neuropsychopharmacology 31*(9), 1928–1937.

Lai, J., Luo, M. C., Chen, Q., Ma, S., Gardell, L. R., Ossipov, M. H., & Porreca, F. (2006). Dynorphin A activates bradykinin receptors to maintain neuropathic pain. *Nature Neuroscience 9*(12), 1534–1540.

Lai, J., Luo, M. C., Chen, Q., & Porreca, F. (2008). Pronociceptive actions of dynorphin via bradykinin receptors. *Neuroscience Letters 437*(3), 175–179.

Lansu, K., Karpiak, J., Liu, J., Huang, X. P., McCorvy, J. D., Kroeze, W. K., ... Roth, B. L. (2017). In silico design of novel probes for the atypical opioid receptor MRGPRX2. *Nature Chemical Biology 13*(5), 529–536.

Lazenka, M. L., Moerke, M. J., Townsend, E. A., Freeman, K. B., Carroll, F. I., & Negus, S. S. (2018). Dissociable effects of the kappa opioid receptor agonist nalfurafine on pain/itch-stimulated and pain/itch-depressed behaviors in male rats. *Psychopharmacology 235*(1), 203–213.

Lee, Y. S., Muthu, D., Hall, S. M., Ramos-Colon, C., Rankin, D., Hu, J., ... Hruby, V. J. (2014). Discovery of amphipathic dynorphin A analogues to inhibit the neuroexcitatory effects of dynorphin A through bradykinin receptors in the spinal cord. *Journal of the American Chemical Society 136*(18), 6608–6616.

Lee, Y. S., Remesic, M., Ramos-Colon, C., Hall, S. M., Kuzmin, A., Rankin, D., ... Hruby, V. J. (2016). Cyclic non-opioid dynorphin A analogues for the bradykinin receptors. *Bioorganic & Medicinal Chemistry Letters 26*(22), 5513–5516.

Leeb-Lundberg, L. M., Marceau, F., Muller-Esterl, W., Pettibone, D. J., & Zuraw, B. L. (2005). International union of pharmacology. XLV. Classification of the kinin receptor family: From molecular mechanisms to pathophysiological consequences. *Pharmacological Reviews 57*(1), 27–77.

Lembo, P. M., Grazzini, E., Groblewski, T., O'Donnell, D., Roy, M. O., Zhang, J., ... Ahmad, S. (2002). Proenkephalin A gene products activate a new family of sensory neuron—specific GPCRs. *Nature Neuroscience 5*(3), 201–209.

Li, Z., He, S. Q., Xu, Q., Yang, F., Tiwari, V., Liu, Q., ... Guan, Y. (2014). Activation of MrgC receptor inhibits N-type calcium channels in small-diameter primary sensory neurons in mice. *Pain 155*(8), 1613–1621.

Li, Z., Tseng, P. Y., Tiwari, V., Xu, Q., He, S. Q., Wang, Y., ... Dong, X. (2017). Targeting human Mas-related G protein-coupled receptor X1 to inhibit persistent pain. *Proceedings of the National Academy of Sciences of the United States of America 114*(10), E1996–E2005.

Lioudyno, M. I., Verbitsky, M., Glowatzki, E., Holt, J. C., Boulter, J., Zadina, J. E., ... Guth, P. S. (2002). The alpha9/alpha10-containing nicotinic ACh receptor is directly modulated by opioid peptides, endomorphin-1, and dynorphin B, proposed efferent cotransmitters in the inner ear. *Molecular and Cellular Neurosciences 20*(4), 695–711.

Lipton, S. A. (2004). Failures and successes of NMDA receptor antagonists: Molecular basis for the use of open-channel blockers like memantine in the treatment of acute and chronic neurologic insults. *NeuroRx 1*(1), 101–110.

Liu, Q., Tang, Z., Surdenikova, L., Kim, S., Patel, K. N., Kim, A., ... Dong, X. (2009). Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell 139*(7), 1353–1365.

Lounsbury, N. (2020). Advances in CXCR7 modulators. *Pharmaceuticals (Basel) 13*(2).

Luo, M. C., Chen, Q., Ossipov, M. H., Rankin, D. R., Porreca, F., & Lai, J. (2008). Spinal dynorphin and bradykinin receptors maintain inflammatory hyperalgesia. *The Journal of Pain 9*(12), 1096–1105.

Lutz, P. E., & Kieffer, B. L. (2013). Opioid receptors: Distinct roles in mood disorders. *Trends in Neurosciences 36*(3), 195–206.

Ma, M., Wang, Z., Wang, J., Wei, S., Cui, J., Wang, Y., Luo, K., Zhao, L., Liu, X., & Wang, R. (2020). Endomorphin analog exhibited superiority in alleviating neuropathic hyperalgesia via weak activation of NMDA receptors. *Journal of Neurochemistry 155*(6), 662–678.

Manglik, A., Lin, H., Aryal, D. K., McCorvy, J. D., Dengler, D., Corder, G., ... Shoichet, B. K. (2016). Structure-based discovery of opioid analgesics with reduced side effects. *Nature 537*(7619), 185–190.

Mansour, A., Hoversten, M. T., Taylor, L. P., Watson, S. J., & Akil, H. (1995). The cloned mu, delta and kappa receptors and their endogenous ligands: Evidence for two opioid peptide recognition cores. *Brain Research 700*(1–2), 89–98.

Martin, W. R. (1979). History and development of mixed opioid agonists, partial agonists and antagonists. *British Journal of Clinical Pharmacology 7*(Suppl. 3), 273S–279S.

Massardier, D., & Hunt, P. F. (1989). A direct non-opiate interaction of dynorphin-(1-13) with the N-methyl-D-aspartate (NMDA) receptor. *European Journal of Pharmacology 170*(1–2), 125–126.

McNeil, B. D., Pundir, P., Meeker, S., Han, L., Undem, B. J., Kulka, M., & Dong, X. (2015). Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature 519*(7542), 237–241.

Medeiros, R., Cabrini, D. A., Ferreira, J., Fernandes, E. S., Mori, M. A., Pesquero, J. B., ... Calixto, J. B. (2004). Bradykinin B1 receptor expression induced by tissue damage in the rat portal vein: A critical role for mitogen-activated protein kinase and nuclear factor-kappaB signaling pathways. *Circulation Research 94*(10), 1375–1382.

Meixiong, J., Vasavda, C., Snyder, S. H., & Dong, X. (2019). MRGPRX4 is a G protein-coupled receptor activated by bile acids that may contribute to cholestatic pruritus. *Proceedings of the National Academy of Sciences of the United States of America 116*(21), 10525–10530.

Melnikova, I. (2010). Pain market. *Nature Reviews. Drug Discovery 9*(8), 589–590.

Menhaji-Klotz, E., Ward, J., Brown, J. A., Loria, P. M., Tan, C., Hesp, K. D., ... Boehm, M. (2020). Discovery of diphenylacetamides as CXCR7 inhibitors with novel beta-arrestin antagonist activity. *ACS Medicinal Chemistry Letters 11*(6), 1330–1334.

Merg, F., Filliol, D., Usynin, I., Bazov, I., Bark, N., Hurd, Y. L., ... Bakalkin, G. (2006). Big dynorphin as a putative endogenous ligand for the kappa-opioid receptor. *Journal of Neurochemistry 97*(1), 292–301.

Meyrath, M., Palmer, C. B., Reynders, N., Vanderplasschen, A., Ollert, M., Bouvier, M., ... Chevigne, A. (2021). Proadrenomedullin N-terminal 20 peptides (PAMPs) are agonists of the chemokine scavenger receptor ACKR3/CXCR7. *ACS Pharmacology & Translational Science 4*(2), 813–823.

Meyrath, M., Szpakowska, M., Zeiner, J., Massotte, L., Merz, M. P., Benkel, T., ... Chevigne, A. (2020). The atypical chemokine receptor ACKR3/CXCR7 is a broad-spectrum scavenger for opioid peptides. *Nature Communications 11*(1), 3033.

Mollereau, C., Parmentier, M., Mailleux, P., Butour, J. L., Moisand, C., Chalon, P., ... Meunier, J. C. (1994). ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. *FEBS Letters 341*(1), 33–38.

Monje, B., Gimenez-Manzorro, A., Ortega-Navarro, C., Herranz-Alonso, A., & Sanjurjo-Saez, M. (2019). Trends in hospital consumption of analgesics after the implementation of a pain performance improvement plan. *Revista Brasileira de Anestesiologia 69*(3), 259–265.

Morgan, M. M., & Christie, M. J. (2011). Analysis of opioid efficacy, tolerance, addiction and dependence from cell culture to human. *British Journal of Pharmacology 164*(4), 1322–1334.

Mullard, A. (2020). FDA approves first GPCR biased agonist. *Nature Reviews. Drug Discovery 19*(10), 659.

Narita, M., & Tseng, L. F. (1998). Evidence for the existence of the beta-endorphin-sensitive "epsilon-opioid receptor" in the brain: The mechanisms of epsilon-mediated antinociception. *Japanese Journal of Pharmacology 76*(3), 233–253.

Navines-Ferrer, A., Serrano-Candelas, E., Lafuente, A., Munoz-Cano, R., Martin, M., & Gastaminza, G. (2018). MRGPRX2-mediated mast cell response to drugs used in perioperative procedures and anaesthesia. *Scientific Reports 8*(1), 11628.

Nibbs, R. J., & Graham, G. J. (2013). Immune regulation by atypical chemokine receptors. *Nature Reviews. Immunology 13*(11), 815–829.

Noble, F., Turcaud, S., Fournie-Zaluski, M. C., & Roques, B. P. (1992). Repeated systemic administration of the mixed inhibitor of enkephalin-degrading enzymes, RB101, does not induce either antinociceptive tolerance or cross-tolerance with morphine. *European Journal of Pharmacology 223*(1), 83–89.

Nothacker, H. P., Reinscheid, R. K., Mansour, A., Henningsen, R. A., Ardati, A., Monsma, F. J., Jr., ... Civelli, O. (1996). Primary structure and tissue distribution of the orphanin FQ precursor. *Proceedings of the National Academy of Sciences of the United States of America 93*(16), 8677–8682.

Odemis, V., Lipfert, J., Kraft, R., Hajek, P., Abraham, G., Hattermann, K., Mentlein, R., & Engele, J. (2012). The presumed atypical chemokine receptor CXCR7 signals through G(i/o) proteins in primary rodent astrocytes and human glioma cells. *Glia 60*(3), 372–381.

O'Hayre, M., Eichel, K., Avino, S., Zhao, X., Steffen, D. J., Feng, X., ... Gutkind, J. S. (2017). Genetic evidence that beta-arrestins are dispensable for the initiation of beta2-adrenergic receptor signaling to ERK. *Science Signaling 10*(484).

Parenti, C., Turnaturi, R., Arico, G., Marrazzo, A., Prezzavento, O., Ronsisvalle, S., ... Pasquinucci, L. (2012). Antinociceptive profile of LP1, a non-peptide multitarget opioid ligand. *Life Sciences 90*(25–26), 957–961.

Pin, J. P., Neubig, R., Bouvier, M., Devi, L., Filizola, M., Javitch, J. A., ... Spedding, M. (2007). International Union of Basic and Clinical Pharmacology. LXVII. Recommendations for the recognition and nomenclature of G protein-coupled receptor heteromultimers. *Pharmacological Reviews 59*(1), 5–13.

Pineyro, G., & Nagi, K. (2021). Signaling diversity of mu- and delta- opioid receptor ligands: Re-evaluating the benefits of beta-arrestin/G protein signaling bias. *Cellular Signalling 80*, 109906.

Planells-Cases, R., Sun, W., Ferrer-Montiel, A. V., & Montal, M. (1993). Molecular cloning, functional expression, and pharmacological characterization of an N-methyl-D-aspartate receptor subunit from human brain. *Proceedings of the National Academy of Sciences of the United States of America 90*(11), 5057–5061.

Podolsky, A. T., Sandweiss, A., Hu, J., Bilsky, E. J., Cain, J. P., Kumirov, V. K., ... Vanderah, T. W. (2013). Novel fentanyl-based dual mu/delta-opioid agonists for the treatment of acute and chronic pain. *Life Sciences 93*(25–26), 1010–1016.

Podvin, S., Yaksh, T., & Hook, V. (2016). The emerging role of spinal dynorphin in chronic pain: A therapeutic perspective. *Annual Review of Pharmacology and Toxicology 56*, 511–533.

Puddinu, V., Casella, S., Radice, E., Thelen, S., Dirnhofer, S., Bertoni, F., & Thelen, M. (2017). ACKR3 expression on diffuse large B cell lymphoma is required for tumor spreading and tissue infiltration. *Oncotarget 8*(49), 85068–85084.

Quinn, K. E., Mackie, D. I., & Caron, K. M. (2018). Emerging roles of atypical chemokine receptor 3 (ACKR3) in normal development and physiology. *Cytokine 109*, 17–23.

Raehal, K. M., Walker, J. K., & Bohn, L. M. (2005). Morphine side effects in beta-arrestin 2 knockout mice. *The Journal of Pharmacology and Experimental Therapeutics 314*(3), 1195–1201.

Rajagopal, S., Kim, J., Ahn, S., Craig, S., Lam, C. M., Gerard, N. P., ... Lefkowitz, R. J. (2010). Beta-arrestin- but not G protein-mediated signaling by the "decoy" receptor CXCR7. *Proceedings of the National Academy of Sciences of the United States of America 107*(2), 628–632.

Richard-Bildstein, S., Aissaoui, H., Pothier, J., Schafer, G., Gnerre, C., Lindenberg, E., Lehembre, F., Pouzol, L., & Guerry, P. (2020). Discovery of the potent, selective, orally available CXCR7 antagonist ACT-1004-1239. *Journal of Medicinal Chemistry 63*(24), 15864–15882.

Robas, N., Mead, E., & Fidock, M. (2003). MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. *The Journal of Biological Chemistry 278*(45), 44400–44404.

Rodriguez-Munoz, M., Sanchez-Blazquez, P., Vicente-Sanchez, A., Berrocoso, E., & Garzon, J. (2012). The mu-opioid receptor and the NMDA receptor associate in PAG neurons: Implications in pain control. *Neuropsychopharmacology 37*(2), 338–349.

Saaber, F., Schutz, D., Miess, E., Abe, P., Desikan, S., Ashok Kumar, P., ... Stumm, R. (2019). ACKR3 regulation of neuronal migration requires ACKR3 phosphorylation, but not beta-arrestin. *Cell Reports 26*(6), 1473–1488 e1479.

Sang, C. N. (2000). NMDA-receptor antagonists in neuropathic pain: Experimental methods to clinical trials. *Journal of Pain and Symptom Management 19*(1 Suppl), S21–S25.

Schmidt, R., Butterworth, J., O'Donnell, D., Santhakumar, V., & Tomaszewski, M. (2009). Cyclic dimers of C-terminal gamma2-MSH analogs as selective antagonists of the human sensory nerve-specific receptor (SNSR-4). *Advances in Experimental Medicine and Biology 611*, 111–112.

Schulz, R., Wuster, M., & Herz, A. (1981). Pharmacological characterization of the epsilon-opiate receptor. *The Journal of Pharmacology and Experimental Therapeutics 216*(3), 604–606.

Sierro, F., Biben, C., Martinez-Munoz, L., Mellado, M., Ransohoff, R. M., Li, M., ... Mackay, F. (2007). Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. *Proceedings of the National Academy of Sciences of the United States of America 104*(37), 14759–14764.

Singla, N., Minkowitz, H. S., Soergel, D. G., Burt, D. A., Subach, R. A., Salamea, M. Y., ... Skobieranda, F. (2017). A randomized, Phase IIb study investigating oliceridine (TRV130), a novel micro-receptor G-protein pathway selective (mu-GPS) modulator, for the management of moderate to severe acute pain following abdominoplasty. *Journal of Pain Research 10*, 2413–2424.

Sjoberg, E., Meyrath, M., Chevigne, A., Ostman, A., Augsten, M., & Szpakowska, M. (2020). The diverse and complex roles of atypical chemokine receptors in cancer: From molecular biology to clinical relevance and therapy. *Advances in Cancer Research 145*, 99–138.

Smit, M. J., Schlecht-Louf, G., Neves, M., van den Bor, J., Penela, P., Siderius, M., ... Mayor, F., Jr. (2021). The CXCL12/CXCR4/ACKR3 axis in the tumor microenvironment: Signaling, crosstalk, and therapeutic targeting. *Annual Review of Pharmacology and Toxicology 61*, 541–563.

Smith, J. S., Lefkowitz, R. J., & Rajagopal, S. (2018). Biased signalling: From simple switches to allosteric microprocessors. *Nature Reviews. Drug Discovery 17*(4), 243–260.

Sotgiu, M. L., Valente, M., Storchi, R., Caramenti, G., & Biella, G. E. (2009). Cooperative N-methyl-D-aspartate (NMDA) receptor antagonism and mu-opioid receptor agonism mediate the methadone inhibition of the spinal neuron pain-related hyperactivity in a rat model of neuropathic pain. *Pharmacological Research 60*(4), 284–290.

Steranka, L. R., Manning, D. C., DeHaas, C. J., Ferkany, J. W., Borosky, S. A., Connor, J. R., ... Snyder, S. H. (1988). Bradykinin as a pain mediator: Receptors are localized to sensory neurons, and antagonists have analgesic actions. *Proceedings of the National Academy of Sciences of the United States of America 85*(9), 3245–3249.

Szpakowska, M., & Chevigne, A. (2016). vCCL2/vMIP-II, the viral master KEYmokine. *Journal of Leukocyte Biology 99*(6), 893–900.

Szpakowska, M., Decker, A. M., Meyrath, M., Palmer, C. B., Blough, B. E., Namjoshi, O. A., & Chevigne, A. (2021). The natural analgesic conolidine targets the newly identified opioid scavenger ACKR3/CXCR7. *Signal Transduction and Targeted Therapy 6*(1), 209.

Szpakowska, M., Dupuis, N., Baragli, A., Counson, M., Hanson, J., Piette, J., & Chevigne, A. (2016). Human herpesvirus 8-encoded chemokine vCCL2/vMIP-II is an agonist of the atypical chemokine receptor ACKR3/CXCR7. *Biochemical Pharmacology 114*, 14–21.

Szpakowska, M., Nevins, A. M., Meyrath, M., Rhainds, D., D'Huys, T., Guite-Vinet, F., ... Chevigne, A. (2018). Different contributions of chemokine N-terminal features attest to a different ligand binding mode and a bias towards activation of ACKR3/CXCR7 compared with CXCR4 and CXCR3. *British Journal of Pharmacology 175*(9), 1419–1438.

Szymaszkiewicz, A., Storr, M., Fichna, J., & Zielinska, M. (2019). Enkephalinase inhibitors, potential therapeutics for the future treatment of diarrhea predominant functional gastrointestinal disorders. *Neurogastroenterology and Motility 31*(4), Article e13526.

Tanaka, K., Kondo, H., Hamamura, K., & Togari, A. (2019). Systemic administration of low-dose naltrexone increases bone mass due to blockade of opioid growth factor receptor signaling in mice osteoblasts. *Life Sciences 224*, 232–240.

Tang, Q., Gandhoke, R., Burritt, A., Hruby, V. J., Porreca, F., & Lai, J. (1999). High-affinity interaction of (des-Tyrosyl)dynorphin A(2-17) with NMDA receptors. *The Journal of Pharmacology and Experimental Therapeutics 291*(2), 760–765.

Tan-No, K., Esashi, A., Nakagawasai, O., Niijima, F., Tadano, T., Sakurada, C., Sakurada, T., Bakalkin, G., Terenius, L., & Kisara, K. (2002). Intrathecally administered big dynorphin, a prodynorphin-derived peptide, produces nociceptive behavior through an N-methyl-D-aspartate receptor mechanism. *Brain Research 952*(1), 7–14.

Tan-No, K., Takahashi, H., Nakagawasai, O., Niijima, F., Sato, T., Satoh, S., Sakurada, S., Marinova, Z., Yakovleva, T., Bakalkin, G., Terenius, L., & Tadano, T. (2005). Pronociceptive role of dynorphins in uninjured animals: N-ethylmaleimide-induced nociceptive behavior mediated through inhibition of dynorphin degradation. *Pain 113*(3), 301–309.

Tarselli, M. A., Raehal, K. M., Brasher, A. K., Streicher, J. M., Groer, C. E., Cameron, M. D., ... Micalizio, G. C. (2011). Synthesis of conolidine, a potent non-opioid analgesic for tonic and persistent pain. *Nature Chemistry 3*(6), 449–453.

Terskiy, A., Wannemacher, K. M., Yadav, P. N., Tsai, M., Tian, B., & Howells, R. D. (2007). Search of the human proteome for endomorphin-1 and endomorphin-2 precursor proteins. *Life Sciences 81*(23–24), 1593–1601.

Toll, L., Khroyan, T. V., Polgar, W. E., Jiang, F., Olsen, C., & Zaveri, N. T. (2009). Comparison of the antinociceptive and antirewarding profiles of novel bifunctional nociceptin receptor/mu-opioid receptor ligands: Implications for therapeutic applications. *The Journal of Pharmacology and Experimental Therapeutics 331*(3), 954–964.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., ... Dingledine, R. (2010). Glutamate receptor ion channels: Structure, regulation, and function. *Pharmacological Reviews 62*(3), 405–496.

Vidal, M. A., Astroza, A., Matus, C. E., Ehrenfeld, P., Pavicic, F., Sanchez, T., ... Figueroa, C. D. (2005). Kinin B2 receptor-coupled signal transduction in human cultured keratinocytes. *The Journal of Investigative Dermatology 124*(1), 178–186.

Walker, J. M., Moises, H. C., Coy, D. H., Baldrighi, G., & Akil, H. (1982). Nonopiate effects of dynorphin and des-Tyr-dynorphin. *Science 218*(4577), 1136–1138.

Waxman, A. R., Juni, A., Kowalczyk, W., Arout, C., Sternberg, W. F., & Kest, B. (2010). Progesterone rapidly recruits female-typical opioid-induced hyperalgesic mechanisms. *Physiology & Behavior 101*(5), 759–763.

Wijtmans, M., Maussang, D., Sirci, F., Scholten, D. J., Canals, M., Mujic-Delic, A., ... Leurs, R. (2012). Synthesis, modeling and functional activity of substituted styrene-amides as small-molecule CXCR7 agonists. *European Journal of Medicinal Chemistry 51*, 184–192.

Wolosker, H. (2006). D-serine regulation of NMDA receptor activity. *Science's STKE 2006*(356), pe41.

Wong, C. S., Cherng, C. H., Luk, H. N., Ho, S. T., & Tung, C. S. (1996). Effects of NMDA receptor antagonists on inhibition of morphine tolerance in rats: Binding at mu-opioid receptors. *European Journal of Pharmacology 297*(1–2), 27–33.

Wong, C. S., Liaw, W. J., Tung, C. S., Su, Y. F., & Ho, S. T. (1996). Ketamine potentiates analgesic effect of morphine in postoperative epidural pain control. *Regional Anesthesia 21*(6), 534–541.

Yaksh, T. L. (1984). Multiple opioid receptor systems in brain and spinal cord: Part I. *European Journal of Anaesthesiology 1*(2), 171–199.

Yu, H., Zhao, T., Liu, S., Wu, Q., Johnson, O., Wu, Z., Zhuang, Z., Shi, Y., Peng, L., He, R., Yang, Y., Sun, J., Wang, X., Xu, H., Zeng, Z., Zou, P., Lei, X., Luo, W., & Li, Y. (2019). MRGPRX4 is a bile acid receptor for human cholestatic itch. *Elife 8*.

Zagon, I. S., Goodman, S. R., & McLaughlin, P. J. (1989). Characterization of zeta (zeta): A new opioid receptor involved in growth. *Brain Research 482*(2), 297–305.

Zhu, J., Chen, C., Xue, J. C., Kunapuli, S., DeRiel, J. K., & Liu-Chen, L. Y. (1995). Cloning of a human kappa opioid receptor from the brain. Life Sciences, 56 (9), PL201-207.

Zou, Y. R., Kottmann, A. H., Kuroda, M., Taniuchi, I., & Littman, D. R. (1998). Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature 393*(6685), 595–599.