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REVIEW

Analgesic synergy between opioid and α_2-adrenoceptors

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Opioid and α_2-adrenoceptor agonists are potent analgesic drugs and their analgesic effects can synergize when co-administered. These supra-additive interactions are potentially beneficial clinically; by increasing efficacy and/or reducing the total drug required to produce sufficient pain relief, undesired side effects can be minimized. However, combination therapies of opioids and α_2-adrenoceptor agonists remain underutilized clinically, in spite of a large body of preclinical evidence describing their synergistic interaction. One possible obstacle to the translation of preclinical findings to clinical applications is a lack of understanding of the mechanisms underlying the synergistic interactions between these two drug classes. In this review, we provide a detailed overview of the interactions between different opioid and α_2-adrenoceptor agonist combinations in preclinical studies. These studies have identified the spinal cord as an important site of action of synergistic interactions, provided insights into which receptors mediate these interactions and explored downstream signalling events enabling synergy. It is now well documented that the activation of both μ and δ opioid receptors can produce synergy with α_2-adrenoceptor agonists and that α_2-adrenoceptor agonists can mediate synergy through either the α_{2A} or the α_{2C} adrenoceptor subtypes. Current hypotheses surrounding the cellular mechanisms mediating opioid–adrenoceptor synergy, including PKC signalling and receptor oligomerization, and the evidence supporting them are presented. Finally, the implications of these findings for clinical applications and drug discovery are discussed.

LINKED ARTICLES

This article is part of a themed section on Opioids: New Pathways to Functional Selectivity. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-2

Abbreviations

CGRP, calcitonin gene-related peptide; DAMGO, [D-Ala^2, NMe-Phe^4, Gly-ol^5]-enkephalin; DeltII, [D-Ala^2]-deltorphin II; DOP, δ opioid; DPDPE, [D-Pen^2, D-Pen^5]enkephalin; DRG, dorsal root ganglia; GIRK, G protein-coupled inwardly-rectifying K^+ channels; i.t., intrathecal; KOP, κ opioid; MOP, μ opioid; PTX, *Pertussis* toxin; SNC80, (±)-4-[(α-R)-α-{2S,5R]-4-allyl-2,5-dimethyl-I-piperazinyl]-3-methoxybenzyl]-N,N-diethyl-benzamide; SP, substance P; ST-91, 2-(2,6-diethylphenylamino)-2-imidazoline HCl; VGCC, voltage-gated Ca^{2+} channel

Introduction

Opioids are widely used to manage all types of pain including acute, cancer, chronic neuropathic and inflammatory pain. Unfortunately, opioid-related adverse effects such as respiratory depression, tolerance, physical dependence and addiction have led to an underutilization of these potent compounds for adequate pain relief. It is clear that current

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opioid therapies could be improved upon, and co-delivery of opioids with other analgesics has shown promise for enhanced analgesic utility.

Agonists acting at α_2-adrenoceptors also have potent spinal and systemic analgesic effects, and are occasionally co-administered as an adjuvant to opioids for acute and chronic pain management. In rodents, co-administration of opioid and α_2-adrenoceptor agonists can produce greater-than-additive (i.e. synergistic) analgesic effects. This adrenoceptor-opioid synergy can potentially improve analgesic efficacy and/or reduce side effects, thus improving the therapeutic index. The clinical application of opioid–adrenoceptor combination therapy is not extensive despite its potential benefits. The development of therapeutic approaches that exploit the combination of opioid and α_2-adrenoceptor agonists is limited, in part, by the lack of mechanistic knowledge on how these drugs interact.

The identification of the receptor subtypes targeted by these drug combinations is important in order to investigate the mechanism mediating opioid–adrenoceptor analgesic synergy. Three distinct receptor subtypes have been identified pharmacologically and correspond to the three cloned opioid receptors: μ , δ and κ opioid (MOP, DOP and KOP) receptors (receptor nomenclature follows Alexander *et al.*, 2013a). All three receptors have the potential to mediate analgesia. MOPs are the most studied opioid receptor because their activation by morphine or other MOP receptor-selective agonists results in a robust analgesic effect (Matthes *et al.*, 1996; Sora *et al.*, 1997). Unfortunately, MOP receptors also mediate unwanted side effects such as analgesic tolerance, physical dependence to morphine and inhibition of gastrointestinal transit (Matthes *et al.*, 1996; Roy *et al.*, 1998; Williams *et al.*, 2013). Currently, clinically available opioid analgesics are MOP receptor agonists. In recent years, DOP receptors have emerged as a promising analgesic target for the treatment of chronic pain (Pradhan *et al.*, 2011). There are also three α_2-adrenoceptor subtypes, α_{2A}, α_{2B} and α_{2C}, which are activated by noradrenaline and adrenaline in the CNS and peripheral nervous system (Bylund *et al.*, 1994). Opioid receptors and α_2-adrenoceptors are preferentially coupled to the *Pertussis* toxin (PTX)-sensitive $G\alpha_{i/o}$ subunit. $G\alpha_{i/o}$ inhibits the production of cAMP by adenylate cyclase, reducing the activation of the cAMP-dependent PKA. Equally important, the free Gβγ counterpart can also act as a signalling molecule and activate downstream signalling pathways, like PLC, or modulate ion channels by activating G-protein coupled inwardly-rectifying K^+ channels (GIRKs) or inhibiting voltage-gated Ca^{2+} channels (VGCCs; channel nomenclature follows Alexander *et al.*, 2013b).

Opioid–adrenoceptor synergistic interactions

The observation that morphine and clonidine administered s.c. could potentiate each other was first reported in mice by Spaulding *et al.* (1979) who suggested that both drugs acted on independent receptor sites through a common pathway. Other studies have also shown that sub-analgesic doses of an α_2-adrenoceptor agonist and an opioid agonist can become analgesic when the two drugs are co-delivered, suggesting that these drugs interact in a synergistic manner (Drasner and Fields, 1988; Plummer *et al.*, 1992; Hao *et al.*, 2000). However, to determine if two drugs interact synergistically, an isobolographic analysis is necessary because it is the most rigorous method available to assess whether the interaction is additive, synergistic or subadditive (see Gessner, 1988; Tallarida, 1992; 2006). This analytical method is employed when two drugs produce a dose-dependent effect and is represented graphically by an isobologram, which was popularized by Loewe (1953). Dose–response curves are determined for each drug alone and for their combination following administration in an equi-effective ratio. A theoretical additive ED_{50} response is calculated based on the single drug dose–response curves and statistically compared with the experimental ED_{50} value of the actual drug combination. If the theoretical and experimental values are significantly different, the combination is said to be either synergistic or antagonistic, depending on the direction of the difference; if not, the interaction is said to be additive. This method is, however, time- and resource-consuming, as several doses must be tested to resolve full dose–response curves for the single drugs and drug combination. This type of analysis is therefore more amenable to preclinical studies and has been used extensively since its first application to investigate opioid–adrenoceptor interactions in rodents by Ossipov *et al.* (1990). Studies compiled in Tables 1 and 2 investigated opioid–adrenoceptor drug interactions in behavioural antinociceptive assays using isobolographic analysis. In order to compare studies side by side and understand their conclusions, critical study parameters are reported in each column.

Although these studies were all performed in rodents, there is no indication that opioid–adrenoceptor interactions are subject to species differences. For example, under similar conditions, the interaction between morphine and clonidine (Table 1) or [D-Pen^2, D-Pen^5]enkephalin (DPDPE) and clonidine (Table 2) is synergistic in both rats and mice. While the majority of studies have been performed on male rats or on mixed male and female cohorts, no effect of sex was apparent on the outcome of the interaction when compared with previously published studies (Fairbanks *et al.*, 2002; Schuster *et al.*, 2013). However, a proper comparison between male and female subjects has yet to be conducted in order to assess the effect of sex on opioid–adrenoceptor synergy.

In contrast, the route of administration is an important determinant of drug interaction, as illustrated in Table 1. Although spinal drug delivery via an implanted cannula was already performed in rats (Yaksh and Rudy, 1976), the development by Hylden and Wilcox (1980) of a simple and rapid method of intrathecal (i.t.) drug delivery in mice greatly contributed to the understanding of opioid–adrenoceptor synergy. Using this spinal drug delivery method, the same group then reported that sub-analgesic doses of an α_2-adrenoceptor agonist potentiated morphine antinociception, and *vice versa* (Hylden and Wilcox, 1983; Wilcox *et al.*, 1987), suggesting that opioid and α_2-adrenoceptor agonists can interact synergistically. Using isobolographic analysis for the first time to evaluate opioid–adrenoceptor interactions, Ossipov *et al.* (1990) observed that clonidine synergy with morphine, fentanyl and meperidine was stronger when the drugs were administered i.t., compared with i.v., leading to the hypothesis that these interactions took place largely at the spinal level. This inference influenced subsequent studies that continued to investigate opioid–adrenoceptor interactions at the spinal level, with a total lack of studies that

Table 1

Interactions between MOP receptor and α_2-adrenoceptor agonists in animal models of nociception

Opioid agonist	Adrenoceptor agonist	Route	Species	Model[a]	Behavioural assay	Interaction	Notes	References
Morphine	Clonidine	i.t.	Rat	Naive	TF	Synergistic	Outcome of interaction depends on route of administration and behavioural assay used. Synergy is stronger at the spinal level.	Ossipov *et al.*, 1990
					HP	Additive		
		i.v.			TF	Additive		
					HP	Synergistic		
		i.t.	Mouse	Naive	SP, TF	Synergistic	Morphine + clonidine interact synergistically in two nociceptive pain models.	Roerig *et al.*, 1992; Chabot-Doré *et al.*, 2013; Roerig, 1995; Roerig and Howse, 1996; Wei *et al.*, 1996; Wei and Roerig, 1998; Fairbanks and Wilcox, 1999; Schuster *et al.*, 2013
		i.t.	Rat, mouse	SNL	TF, MS	Synergistic	Synergy persists in neuropathic pain model.	Ossipov *et al.*, 1997; Fairbanks *et al.*, 2000a
		i.t.	Rat	Formalin assay	FT	Synergistic	Synergy observed in formalin pain model.	Przesmycki *et al.*, 1997
		i.p.	Mouse	SPARC-null	TS	Synergistic	Synergy ameliorates signs of axial pain in a mouse model of low back pain.	Tajerian *et al.*, 2012
		i.t.	Mouse	Morphine tolerant	TF	Additive or Synergistic	Interaction depends on the morphine tolerance and drug combination paradigms used.	Roerig, 1995; Fairbanks and Wilcox, 1999
		i.t.	Mouse	PTX	TF	Synergistic	Synergy is not mediated by a PTX-sensitive G-protein coupled mechanism.	Roerig and Howse, 1996; Wei *et al.*, 1996
		i.t.	Mouse	ω-agatoxin IVA	TF	Additive	Synergy involves P-type VGCC.	Roerig and Howse, 1996
		i.t.	Mouse	PTX + ω-conotoxin	TF	Additive	Morphine–clonidine synergy requires both $G_{i/o}$ protein and N-type VGCC activation.	Wei *et al.*, 1996
		i.t.	Mouse	Chelerythrine, Calphostin C	TF	Additive	PKC, but not PKA, inhibition blocks morphine clonidine synergy.	Wei and Roerig, 1998
		i.t.	Mouse	H-89	TF	Synergistic		
		i.t.	Mouse	PKCε-KO	TF	Synergistic	Morphine + clonidine synergy is not PKCε-dependent.	Schuster *et al.*, 2013
		i.t.	Mouse	DOP-KO	SP	Synergistic	Morphine + clonidine synergy does not require DOP.	Chabot-Doré *et al.*, 2013

	Noradrenaline	i.t.	Mouse	Naive	SP	Synergistic	Morphine + noradrenaline interact synergistically.	Roerig *et al.*, 1992
	ST-91	i.t.	Rat	Naive	HP	Synergistic	Morphine + ST-91 interact synergistically.	Monasky *et al.*, 1990
	Moxonidine	i.t.	Mouse	Naive	SP	Synergistic	Morphine + moxonidine interaction is synergistic in both naive and neuropathic pain model.	Fairbanks *et al.*, 2000a, b
				SNL	MS	Synergistic		
	Brimonidine	i.t.	Mouse	WT, PKCε-KO	TF	Synergistic	PKCε is not necessary for morphine-brimonidine interaction.	Schuster *et al.*, 2013
DAMGO	Clonidine	i.t.	Mouse	Naive	TF	Synergistic	DAMGO + clonidine interaction is assay-dependent.	Roerig, 1995
					SP	Antagonistic or additive		Roerig *et al.*, 1992; Chabot-Doré *et al.*, 2013
	Noradrenaline	i.t.	Mouse	Naive	SP	Antagonistic	DAMGO + noradrenaline interaction is sub-additive	Roerig *et al.*, 1992
	Moxonidine	i.t.	Mouse	Naive	SP	Antagonistic	DAMGO + moxonidine interaction is sub-additive	Fairbanks *et al.*, 2000b
	Brimonidine	i.t.	Mouse	Naive	SP	Synergistic	DAMGO + brimonidine synergy is mediated through the α_{2A}-adrenoceptor.	Stone *et al.*, 1997
				D79N–α_{2A}-adrenoceptor-	SP	Additive		
Endomorphin	Clonidine	i.t.	Mouse	Naive	TF	Synergistic	PKCε is not required for endomorphin-mediated interactions with clonidine or brimonidine.	Schuster *et al.*, 2013
				PKCε-KO				
	Brimonidine	i.t.	Mouse	Naive	TF	Synergistic		
				PKCε-KO				
Fentanyl	Clonidine	i.v.	Rat	Naive	TF, HP	Additive	Outcome of interaction depends on route of administration.	Ossipov *et al.*, 1990
		i.t.			TF, HP	Synergistic		
Meperidine	Clonidine	i.v.	Rat	Naive	TF, HP	Additive	Outcome of interaction depends on route of administration.	Ossipov *et al.*, 1990
		i.t.			TF	Synergistic		

[a]Model: includes genetically modified animals, pain states or drug treatments other than the opioid– adrenoceptor agonist combination.
FT, formalin test; HP, hot plate assay; KO, knockout; MS, mechanical sensitivity; SNL, spinal nerve ligation; TF, tail flick assay; TS, tail suspension.

Table 2

Interactions between DOP receptor and α_2-adrenoceptor agonists in animal models of nociception

Opioid agonist	Adrenoceptor agonist	Route	Specie	Model[a]	Behavioural assay	Interaction	Notes	References
DeltII	Clonidine	i.t.	Mouse	Naive	TF, SP	Synergistic	DeltII + clonidine interact synergistically in two nociceptive pain models.	Overland *et al.*, 2009; Schuster *et al.*, 2013; Chabot-Doré *et al.*, 2013
		i.t.	Mouse	DOP-KO	SP	No interaction	DeltII–clonidine synergy requires DOP.	Chabot-Doré *et al.*, 2013
		i.t.	Mouse	Chelerythrine	TF	Additive	DeltII–clonidine synergy is PKCε-dependent.	Overland *et al.*, 2009
				PKCε-KO	TF	Additive		Schuster *et al.*, 2013
	Moxonidine	i.t.	Mouse	Naive	SP	Synergistic	DeltII–moxonidine synergistic interaction is dependent on α_{2C}-, not α_{2A}-adrenoceptors.	Fairbanks *et al.*, 2000b; Fairbanks *et al.*, 2002
				α_{2C}-adrenoceptor KO		Additive		
				D79N-α_{2A}-adrenoceptor		Synergistic		
	ST-91	i.t.	Mouse	WT; D79N-α_{2A}; α_{2C}-KO	SP	Synergistic	DeltII–ST-91 synergy does not require α_{2C} or α_{2A}-adrenoceptors.	Stone *et al.*, 2007
	Brimonidine	i.t.	Mouse	Naive	TF	Synergistic	Inhibiting PKCα/β or PKCδ does not impair DeltII–brimonidine synergy, but inhibiting or genetically deleting PKCε does.	Schuster *et al.*, 2013
				PKCε-KO, PKCε inhibitor		Additive		
				PKCα/β inhibitor, PKCδ inhibitor		Synergistic		
		i.t.	Mouse	Naive	SP	Synergistic	DeltII–brimonidine synergy requires α_{2A}-adrenoceptors.	Stone *et al.*, 1997
				D79N-α_{2A}-adrenoceptor		Additive		
DPDPE	Clonidine	i.t.	Rat, Mouse	Naive	TF, SP	Synergistic	DPDPE + clonidine interact synergistically in two nociceptive pain models.	Roerig *et al.*, 1992; Ossipov *et al.*, 1990
	Noradrenaline	i.t.	Mouse	Naive	SP	Synergistic	DPDPE + noradrenaline interact synergistically.	Roerig *et al.*, 1992
	Brimonidine	i.t.	Mouse	Naive	SP	Synergistic	MOP is not necessary for DPDPE–brimonidine interaction	Guo *et al.*, 2003
				MOP-KO		Synergistic		
SNC80	Clonidine	i.t.	Mouse	Naive	TF	Synergistic	PKCε is required for SNC80-mediated synergistic interaction with clonidine or brimonidine.	Schuster *et al.*, 2013
				PKCε-KO		Additive		
	Brimonidine	i.t.	Mouse	Naive	TF	Synergistic		
				PKCε-KO		Additive		

[a]Model: includes genetically modified animals, pain states or drug treatments other than the opioid–adrenergic combination.
HP, hot plate assay; KO, knockout; MS, mechanical sensitivity; SNL, spinal nerve ligation; TF, tail flick assay.

looked for equivalent synergy at other sites of actions. For example, despite an extensive co-expression of MOP receptors and α_2-adrenoceptors in locus coerulus neurons, electrophysiological recordings revealed only an additive interaction among these receptors (Stone and Wilcox, 2004). Although synergistic interactions are observed when drugs are administered systemically (Ossipov *et al.*, 1990; Tajerian *et al.*, 2012), there is little evidence suggesting that synergy occurs outside the spinal cord. Further studies are therefore necessary to explore the full potential of other routes of administration that are more practical than spinal delivery for the treatment of chronic pain.

The use of different combinations of receptor subtype-selective agonists and genetically altered mouse models have allowed the identification of two opioid receptor subtypes, MOP and DOP, and two adrenoceptor subtypes, α_{2A} and α_{2C}, capable of producing synergistic opioid–adrenoceptor interactions. The MOP- and DOP receptor-mediated interactions with different α_2-adrenoceptor agonists have been collected in Tables 1 and 2, respectively. This review will not address KOP receptor-mediated interactions as only one study tested and confirmed the synergistic interaction between a KOP receptor agonist and clonidine (Roerig, 1995).

Pharmacokinetic mechanisms

Pharmacokinetic interactions could contribute to opioid–adrenoceptor synergy. Spinally administered α_2-adrenoceptor agonists like clonidine exert a vasoconstrictive effect (Asada and Lee, 1992; Iida *et al.*, 1999) that might cause reduced drug clearance from the site of administration. Therefore, it is possible that synergistic interactions involving clonidine could be due to an enhanced effect of the other drug resulting from the extension of its duration of action at the site of administration. If synergistic opioid–adrenoceptor interactions were the result of such pharmacokinetic mechanisms, we would expect clonidine to synergize with all opioid agonists; however, this is not the case. Clonidine, along with noradrenaline and moxonidine, are additive or antagonistic with [D-Ala^2, NMe-Phe^4, Gly-ol^5]-enkephalin (DAMGO) in the substance P (SP) behavioural assay (Roerig *et al.*, 1992; Fairbanks *et al.*, 2000b; Chabot-Doré *et al.*, 2013). Additionally, although clearance of morphine from the spinal cord is not affected by the co-delivery of the α_2-adrenoceptor agonist, 2-(2,6-diethylphenylamino)-2-imidazoline HCl (ST-91; Monasky *et al.*, 1990), morphine and ST-91 produce antinociceptive synergy (Yasuoka and Yaksh, 1983; Monasky *et al.*, 1990; Nagasaka and Yaksh, 1990). Therefore, it is unlikely that pharmacokinetic effects of α_2-adrenoceptor agonists contribute significantly to opioid–adrenoceptor synergy at the spinal level.

Pharmacodynamic mechanisms

Synergistic interactions between opioid and α_2-adrenoceptor agonists could be mediated by either intercellular or intracellular mechanisms, depending on the location of the receptors involved. Drugs delivered into the thecal space at the lumbar level act on neurons intrinsic to the spinal cord as well as on axon terminals originating from dorsal root ganglion (DRG) neurons or from descending modulatory pathways. These structures are important in nociceptive signal processing and have opioid and adrenoceptors capable of inhibiting the transmission of afferent nociceptive signals. α_{2A}-adrenoceptors and DOP receptors have been reported to co-localize primarily on primary afferent terminals, specifically peptidergic afferents expressing the neuropeptides SP and/or calcitonin gene-related peptide (CGRP), in the superficial dorsal horn of the spinal cord (Dado *et al.*, 1993; Stone *et al.*, 1998; Zhang *et al.*, 1998; Bao *et al.*, 2003; Riedl *et al.*, 2009). However, evidence also indicates that a portion of these receptors reside on cells intrinsic to the spinal cord (Cheng *et al.*, 1997; Shi *et al.*, 1999; Cahill *et al.*, 2001a). In addition, α_{2A}-adrenoceptors are also expressed on descending noradrenergic axon terminals where they act as autoreceptors to inhibit noradrenaline release (Li *et al.*, 2000; Gilsbach *et al.*, 2009). MOP receptors are located in both primary afferent neurons and spinal cord neurons receiving input from primary afferents (Arvidsson *et al.*, 1995; Aicher *et al.*, 2000; Gupta *et al.*, 2010; Wang *et al.*, 2010; Beaudry *et al.*, 2011; He *et al.*, 2011). The α_{2C} adrenoceptors are known to be located primarily on spinal cord interneurons and to a much lesser degree on incoming primary afferents (Stone *et al.*, 1998; Olave and Maxwell, 2002; 2003). Drugs acting on receptors located within the same cell could produce interactions at the level of intracellular signalling cascades, whereas drugs acting on receptors located in two different cells will presumably act independent of the intracellular pathways activated upon receptor binding. In this case, the synergistic interaction would not be due to an intracellular signalling interaction, but rather to coincident inhibition of two neurons in series in the same anatomical pathway or via a retrograde feedback mechanism.

Synergistic interactions as a functional consequence of opioid–adrenoceptor heteromerization

One of the leading hypotheses explaining these synergistic interactions is the formation of opioid–adrenoceptor heteromeric receptor complexes. Upon oligomerization, the pharmacological properties of heteromeric receptors can differ from those of their respective monomers. Many aspects of GPCR function can be affected by oligomerization, including synthesis, surface expression, ligand binding, receptor activation, signal transduction and internalization, all of which may have important functional implications (Terrillon and Bouvier, 2004). The first step to investigate this hypothesis is to identify candidate receptors that interact synergistically. Second, the ability of candidate receptor pairs to physically interact in native cells must be demonstrated. Finally, elucidating the mechanism by which the heteromers mediate the synergistic interaction would confirm the hypothesis. As the functional consequences of heteromer interactions are numerous and complex, several avenues can be tested before determining the mechanism. The current evidence supporting or invalidating a candidate mechanism for a heteromeric interaction based on the criteria mentioned earlier are discussed later for some opioid–adrenoceptor pairs.

MOP receptor-mediated synergistic interactions

Synergy between MOP and opioid–adrenoceptor agonists is well documented (Table 1). Morphine, which requires the

MOP receptor for analgesic activity (Matthes *et al.*, 1996; Sora *et al.*, 1997), synergizes at the spinal level with several α_2-adrenoceptor agonists including clonidine (Ossipov *et al.*, 1990; Roerig *et al.*, 1992; Fairbanks and Wilcox, 1999; Chabot-Doré *et al.*, 2013), brimonidine (Stone *et al.*, 1997; Schuster *et al.*, 2013), moxonidine (Fairbanks *et al.*, 2000b), ST-91 (Monasky *et al.*, 1990) and noradrenaline (Roerig *et al.*, 1992). As with morphine, the MOP receptor agonist, endomorphin II, also interacts synergistically with clonidine and brimonidine (also known as UK 14 304) at the spinal level (Schuster *et al.*, 2013). In contrast, the MOP receptor agonist, DAMGO, does not mimic the results observed with morphine; instead, DAMGO yields a sub-additive interaction with moxonidine and noradrenaline (Roerig *et al.*, 1992; Fairbanks *et al.*, 2002), either a sub-additive, synergistic, or additive interaction with clonidine depending on the assay used (Roerig *et al.*, 1992; Roerig, 1995; Chabot-Doré *et al.*, 2013), and a synergistic interaction with brimonidine (Stone *et al.*, 1997). These results indicate that the occurrence and mechanism of synergy between MOP receptor and α_2-adrenoceptor agonists depends on the assay, as well as the specific ligand combination used.

Interactions between MOP and DOP receptors have been shown to modulate the response to morphine *in vivo* and *in vitro* (Costantino *et al.*, 2012). For example, in cells co-expressing MOP and DOP receptors, the signalling output following morphine treatment is stronger than in cells expressing only MOP receptors (Yekkirala *et al.*, 2010). Furthermore, DOP receptor-selective ligands can potentiate morphine analgesia *in vivo* (Gomes *et al.*, 2004; Schuster *et al.*, 2015, provisionally accepted for publication in *BJP*) and the absence of DOP receptors blocks the development of tolerance to morphine (Zhu *et al.*, 1999). Morphine also acts on MOP receptors to up-regulate the expression of surface DOP receptors (Cahill *et al.*, 2001b; Morinville *et al.*, 2003; Gendron *et al.*, 2006). Despite the involvement of DOP receptors in a range of morphine's effects, DOP receptors are not necessary for the synergistic interaction between morphine and clonidine (Chabot-Doré *et al.*, 2013). Therefore, MOP receptor-mediated synergy appears to be distinct from DOP receptor-mediated synergy.

DOP receptor-mediated synergistic interactions

The reports that DOP receptor agonists had antinociceptive effects when administered spinally (Hylden and Wilcox, 1982; Tung and Yaksh, 1982) raised the possibility that these drugs could also interact synergistically with α_{2A}-adrenoceptor agonists. Roerig *et al.* (1992) were first to report synergistic interactions involving DOP receptor agonists. Since then, spinal analgesic synergy between DOP receptors and α_2-adrenoceptors has been observed with several different agonist combinations (Table 2). The DOP receptor agonists [D-Ala^2]-deltorphin II (DeltII), DPDPE and (±)-4-[(α-R)-α-{2S,5R]-4-allyl-2,5-dimethyl-I-piperazinyl]-3-methoxybenzyl]-N,N-diethyl-benzamide (SNC80) all produce antinociceptive synergy when co-delivered spinally with the α_2-adrenoceptor agonists clonidine and brimonidine (Ossipov *et al.*, 1990; Roerig *et al.*, 1992; Stone *et al.*, 1997; Guo *et al.*, 2003; Overland *et al.*, 2009; Schuster *et al.*, 2013). Additionally, DeltII synergizes with the α_2-adrenoceptor agonists moxonidine (Fairbanks *et al.*, 2000b; 2002) and ST-91 (Stone *et al.*, 2007). The involvement of DOP receptors in these interactions has come into question because these DOP agonists continue to have some antinociceptive activity in DOP receptor knockout mice (Scherrer *et al.*, 2004; van Rijn *et al.*, 2012). However, other studies have supported the role of DOP receptors in the observed interactions by showing that synergy between DPDPE and brimonidine persists in MOP receptor-knockout mice (Guo *et al.*, 2003), and that DOP receptors are required for synergy between DeltII and clonidine (Chabot-Doré *et al.*, 2013). These studies indicate that synergy with α_2-adrenoceptors can be obtained by activating either MOP or DOP receptors, confirming that at least two different opioid receptors can mediate synergy at the spinal level. Whether these pathways share a common mechanism remains an open question.

α_2-adrenoceptor subtypes mediating synergistic interaction with opioids

Because of the general lack of subtype selectivity of α_2-adrenoceptor agonists and antagonists, the identification of α_2-adrenoceptor subtypes mediating opioid–adrenoceptor synergistic interactions was difficult before the advent of genetically altered mice (Tables 1 and 2). The generation of a mouse line with a dysfunctional α_{2A}-adrenoceptor, the D79N-α_{2A} receptor, provided evidence that this adrenoceptor subtype mediated the antinociceptive, hypotensive and sedative effects of α_2-adrenoceptor agonists (MacMillan *et al.*, 1996; Lakhlani *et al.*, 1997). Three opioid–adrenoceptor combinations were then tested in these mice to determine the role of α_{2A}-adrenoceptors in opioid–adrenoceptor synergy. For brimonidine and DeltII or DAMGO, the synergistic interaction was mediated by α_{2A}-adrenoceptors (Stone *et al.*, 1997), as mice expressing the dysfunctional α_{2A}-adrenoceptors exhibited no analgesic synergy. In contrast, the α_{2A}-adrenoceptor was not necessary for the synergistic interaction between moxonidine and DeltII, which was instead mediated by the α_{2C}-adrenoceptor (Fairbanks *et al.*, 2002). Another α_2-adrenoceptor agonist, ST-91, interacts synergistically with DeltII in mice with the mutant D79N-α_{2A}-adrenoceptor or in α_{2C}-adrenoceptor-KO mice (Stone *et al.*, 2007). This outcome suggests that either ST-91 is versatile and can produce synergy via either α_{2A} or α_{2C}-adrenoceptors, or that the α_{2B} receptors mediate this interaction. Thus, depending on the adrenoceptor agonist used, it appears that either α_{2A} or α_{2C} adrenoceptors can mediate synergistic interactions.

Cellular signalling mechanisms underlying opioid–adrenoceptor synergy

Little is known about the downstream signalling mechanism(s) involved in the synergistic interaction between opioid and adrenoceptor agonists. To date, a few studies attempted to identify a signalling pathway specific to the synergistic interaction. Inhibition of $G_{i/o}$ with PTX is not sufficient to turn the synergistic interaction into an additive one (Roerig and Howse, 1996; Wei *et al.*, 1996). Furthermore, increasing cAMP levels or inhibiting PKA affected neither the synergistic interaction between morphine and clonidine nor that between DeltII and clonidine; this result rules out PKA, an important downstream target of $G\alpha_{i/o}$, from playing a role

in the synergy (Wei and Roerig, 1998; Overland *et al.*, 2009). While the inhibition of L- or N-type VGCC did not affect synergy, the simultaneous inactivation of $G\alpha_{i/o}$ and N-type VGCC rendered the morphine–clonidine interaction additive (Wei *et al.*, 1996). Moreover, blocking P-type VGCCs changed the interaction from synergistic to additive (Roerig and Howse, 1996). These results suggest that N- and P-type VGCCs are involved in synergy, but their exact role remains unclear.

PKC activation has been specifically implicated in opioid–adrenoceptor synergistic interactions. Inhibitors of PKC administered i.t. have been shown to block the synergistic interaction between morphine and clonidine (Wei and Roerig, 1998) or DeltII and clonidine (Overland *et al.*, 2009), but not the antinociceptive effect of any one drug given alone.

PKCε activation is required for DOP receptor-mediated synergistic interactions

Using an assay measuring K^+-induced CGRP release from spinal cord slices, Overland *et al.* (2009) were able to reproduce DeltII-clonidine synergy and its blockade by PKC inhibitors. As Gβγ can activate PLC, which then generates second messengers capable of activating PKCs, the authors tested if this pathway was involved. The effect of DeltII, clonidine and their combination was lost when PLC was inhibited. This result suggests that PLC contributes to the antinociceptive effect of DeltII and clonidine and that it is upstream of PKC in mediating opioid–adrenoceptor synergy.

Several isoforms of PKC can be found in DRG neurons, including, but not limited to, α, β, γ, δ and ε (Cesare *et al.*, 1999). PKC signalling is very complex, and different isoforms are not always activated by the same stimuli, nor do they signal to the same downstream effectors (Newton and Messing, 2010), making it difficult to know how PKC, or which PKC isoform, is involved in synergy between DOP and α_{2A}-adrenoceptor agonists. Work by Schuster *et al.* (2013) using isoform-specific peptide inhibitors and the ε isoform of PKC (PKCε)-knockout mice has shown that interactions involving α_{2A}-adrenoceptors and DOP receptors specifically require PKCε. $PLC_{\beta3}$ is directly upstream of PKCε in primary afferent neurons (Joseph *et al.*, 2007), and $G\alpha_i$-associated Gβγ and $G\alpha_q$ subunits can synergistically activate $PLC_{\beta3}$ (Philip *et al.*, 2010; Rebres *et al.*, 2011). Considering this, activation of either α_{2A} adrenoceptors or DOP receptors alone might be insufficient to induce activation of PKCε. Instead, co-activation of different Gα subunits by α_{2A}-adrenoceptors and DOP receptors might be required for signalling to PKCε. Support for this hypothesis comes from the finding that activation or inhibition of PKCε at the spinal level is insufficient to change potency of DeltII or brimonidine delivered singly (Schuster *et al.*, 2013). This result rules out the possibility that one agonist activates PKCε leading to a subsequent increase in potency of the other agonist, and suggests instead that receptor co-activation is necessary for involvement of PKCε in enhanced analgesia.

It is not clear how PKCε is involved in the enhanced analgesia produced upon co-delivery of an α_{2A}-adrenoceptor agonist with a DOP receptor agonist. There is evidence that PKCε plays a role in regulation of trafficking of vesicles and associated receptors to the cell surface (Csukai *et al.*, 1997; Chou *et al.*, 2010). Although these studies did not investigate trafficking of α_{2A}-adrenoceptors and DOP receptors, it is possible that PKCε may alter surface availability of these receptors via similar mechanisms. PKCε may also play a role in induction of functional competence of DOP receptors by certain stimuli. In primary afferent neurons, bradykinin can induce increased functional competence of DOP receptors in a PKC-dependent manner (Patwardhan *et al.*, 2005; Rowan *et al.*, 2009), although the specific PKC isoform involved was not evaluated in these studies.

The necessity for receptor co-activation in order to trigger a signalling cascade supports the finding that the receptors are co-expressed in the same cells (Riedl *et al.*, 2009). DOP receptors and α_{2A}-adrenoceptors can both exert their analgesic action by inhibiting transmitter release from primary afferent terminals (Glaum *et al.*, 1994; Kawasaki *et al.*, 2003). Evidence suggests that the synergistic interaction between DeltII and clonidine is mediated at the level of the primary afferent nerve terminal; for example, this drug combination synergistically inhibited KCl-induced CGRP release from spinal cord slices (Overland *et al.*, 2009) and spinal synaptosomes (Riedl *et al.*, 2009). These outcomes indicate that peptidergic primary afferent neurons are a site of action of opioid–adrenoceptor synergy. We have demonstrated that DOP receptors and α_{2A}-adrenoceptors are co-expressed in primary afferent neurons and highly co-localize in SP-immunoreactive neurons and isolated nerve terminals (Riedl *et al.*, 2009). Although the localization of DOP receptors in SP-positive neurons is debated (Scherrer *et al.*, 2009; Wang *et al.*, 2010), the above-mentioned physiological and anatomical evidence supports the presence of DOP receptors and α_{2A}-adrenoceptors in peptidergic neurons, where they would be positioned to inhibit neurotransmitter release in a synergistic manner.

It is hypothesized that this receptor pair forms hetero-oligomers that mediate opioid–adrenoceptor synergy *in vivo*. In HEK cells co-expressing epitope-tagged DOP receptors and α_{2A}-adrenoceptors, these receptors were demonstrated to be within 100 Å of each other as shown by FRET and part of the same protein complex as shown by co-immunoprecipitation assay (Rios *et al.*, 2004). Thus, DOP receptors and α_{2A}-adrenoceptors are co-expressed in DRG neurons and may be close enough to interact physically *in vivo*, as well as in expression systems. The link between the DOP-α_{2A}-adrenoceptor heteromer and analgesic synergy remains to be elucidated and is the focus of current research. It is possible that, as a result of heteromer formation, the co-activation of DOP receptor and α_{2A}-adrenoceptor recruits distinct downstream signalling effectors resulting in the activation of PKCε, which would in turn mediate the analgesic synergy.

Molecular mechanisms underlying MOP receptor-mediated synergistic interactions

As interactions between MOP receptors and α_2-adrenoceptors are dependent on the specific agonists present, it appears that the mechanisms underlying these interactions may be modulated by ligand-induced bias in downstream signalling. Differences between morphine- and DAMGO-induced signalling through MOP receptors have been documented both in single drug and drug interaction studies. Desensitization of MOP receptors by morphine is PKC-dependent, whereas

desensitization by DAMGO is β-arrestin-dependent (Johnson *et al.*, 2006; Chu *et al.*, 2008). Additionally, while morphine did not cross-desensitize the effects of clonidine in a primary sensory neuron culture model, DAMGO caused both cross-desensitization and co-internalization of α_2-adrenoceptors with MOP receptors (Tan *et al.*, 2009). The lack of a synergistic interaction *in vivo* between DAMGO and clonidine (Roerig *et al.*, 1992; Chabot-Doré *et al.*, 2013) could be a consequence of this cross-desensitization.

Interestingly, the synergistic interaction arising from the combination of morphine and clonidine is also PKC-dependent (Wei and Roerig, 1998). PKC activation is not a typical signalling event downstream of the PTX-sensitive $G\alpha_{i/o}$ signalling pathway usually associated with the activation of opioid and adrenoceptors. As uncoupling the $G\alpha_{i/o}$ subunit decreases morphine and clonidine potency without disrupting their synergistic interaction (Roerig and Howse, 1996; Wei *et al.*, 1996), a signalling pathway independent of those normally activated by either drug alone may mediate morphine–clonidine synergy. Studies in PKCε-knockout mice indicate that this isoform mediates neither spinal morphine analgesia nor interactions between morphine and α_{2A}-adrenoceptor agonists (Schuster *et al.*, 2013), but involvement of other isoforms has not been evaluated.

MOP receptors and α_{2A}-adrenoceptors have been identified as receptor subtypes capable of mediating synergistic interactions (Stone *et al.*, 1997; Chabot-Doré *et al.*, 2013). Given the distribution of these receptors in DRG and spinal cord neurons, their co-expression is likely. For example, peptidergic nociceptors have been found to express MOP receptors (Wang *et al.*, 2010) and α_{2A}-adrenoceptors (Stone *et al.*, 1998; Riedl *et al.*, 2009). However, the only direct assessment of their co-expression in the spinal cord showed that the immunoreactivity for MOP receptors and for α_{2A}-adrenoceptors did not overlap (Riedl *et al.*, 2009). It is possible that the antibodies used in this study each recognized only a subset of their target receptors (e.g. a splice variant). Using different primary antibodies raised against MOP receptors and α_{2A}-adrenoceptors, other immunohistochemical studies showed different labelling patterns in the spinal cord (Rosin *et al.*, 1993; Arvidsson *et al.*, 1995) and suggest that these receptors could still be co-expressed. Furthermore, Tan *et al.* (2009) showed an extensive colocalization of immunoreactive MOP receptors and α_{2A}-adrenoceptors at the cell surface and in neuronal projections of cultured DRG neurons that was associated with functional cross-talk between the receptors. These data, together with the observations that morphine and clonidine can both inhibit C fibre-evoked responses (Sullivan *et al.*, 1987) and capsaicin-induced glutamate release from spinal cord synaptosomes (Li and Eisenach, 2001), make a strong case for the co-expression and functional interaction of MOP receptor and α_{2A}-adrenoceptors in nociceptors. Thus, MOP-mediated synergistic interactions with α_2-adrenoceptor agonists may result from interactions between MOP receptors and α_{2A}-adrenoceptors within the same cells, which could be mediated by the heteromerization of these receptors.

In heterologous expression systems, MOP receptors and α_{2A}-adrenoceptors interact physically and functionally. When co-expressed, the receptors are in close enough proximity to produce the emission of bioluminescence resonance energy transfer (BRET) signals (Jordan *et al.*, 2003) or FRET (Vilardaga *et al.*, 2008), and they co-immunoprecipitate in the same protein complex (Jordan *et al.*, 2003; Zhang and Limbird, 2004). This direct interaction between MOP receptors and α_{2A}-adrenoceptors has important functional consequences. Vilardaga *et al.* (2008) studied the effect of ligand-induced cross-conformational change between MOP receptors and α_{2A}-adrenoceptors and how it modulates coupling to downstream signalling. They showed that morphine reduced noradrenaline-mediated conformational changes in the α_{2A}-adrenoceptors and downstream activation of $G\alpha_i$ induced by noradrenaline. Furthermore, the inhibition of $G\alpha_i$ signalling was strongly correlated with the inhibition of the ERK1/2 signalling pathway, suggesting that the activation state of MOP receptors influences the output of the α_{2A}-adrenoceptors. This study hence demonstrated the ability of MOP receptors to allosterically modulate α_{2A}-adrenoceptors, but did not test the reverse, that is the ability of α_{2A}-adrenoceptors to allosterically modulate MOP receptors, which does not exclude the possibility that allosteric modulations work symmetrically within this receptor pair. In the presence of a single agonist, MOP receptors and α_{2A}-adrenoceptors normally activate MAPK signalling pathways. Interestingly, the observation that $G\alpha_i$ signalling output decreases when both receptors are occupied by an agonist is in agreement with the decrease in GTPγS binding and phosphorylation of MAPK upon morphine and clonidine treatment in cells expressing both MOP receptors and α_{2A}-adrenoceptors (Jordan *et al.*, 2003). Therefore, the suppression of $G\alpha_i$ protein coupling and downstream signalling may indicate that MOP-α_{2A}-adrenoceptor heteromers switch to a new signalling pathway when the receptors are co-activated. As PKC-dependent synergistic interactions are obtained when these receptors are co-activated, MOP-α_{2A}-adrenoceptor heteromers are probably switching to a signalling pathway activating downstream PKCs.

Clinical applications of opioid–adrenoceptor synergy

Due to the strong synergistic interaction observed when opioid and adrenoceptor agonists are administered spinally, the evidence suggests that treatments would be most effective if delivered spinally. Local administration of the agonists at the site of action circumvents action of opioids and α_2-adrenoceptor agonists in the periphery and in supraspinal areas where they mediate undesired side effects such as constipation, sedation, hypotension and respiratory depression. For example, the spinal co-administration of sub-analgesic doses of morphine and clonidine produced an analgesic effect without lowering blood pressure, which would normally be observed at higher doses in rats (Loomis *et al.*, 1988). However, i.t. or epidural drug delivery methods are not practical for most patients, a fact which represents an obstacle to the rapid translation of the preclinical knowledge summarized in this review. The antinociceptive synergy between morphine and clonidine is maintained when drugs are administered systemically, and side effects are not potentiated by the combination (Ossipov *et al.*, 1990; Tajerian *et al.*, 2012). As adrenoceptor agonists are already available and approved for systemic delivery, the preclinical findings

suggest that they could be used in combination with opioids to obtain augmented analgesia.

The synergistic interactions observed in rodents support the notion that combination therapy can be beneficial for pain management by reducing the doses of drugs needed to produce analgesia, increasing efficacy or reducing side effects compared with single drug therapy. However, the clinical validation of these findings is lagging behind. Currently, we are aware of only one study in the clinical literature applying isobolographic analysis to evaluate interactions between opioid and adrenoceptor analgesics in humans, which showed an interaction that did not differ significantly from additivity, despite a trend towards synergy (Eisenach *et al.*, 1994). Several factors are likely to contribute to this gap in the literature including the cumbersome and costly nature of the necessary studies. A standard isobolographic analysis requires an objective measure of pain intensity and the generation of at least three dose–response curves: one for each single drug, and one for a combination of the two drugs given in a fixed ratio determined by the relative potency of each single drug. Such a study would be a significantly greater undertaking than a single-drug clinical trial. Clinical studies of opioid–adrenoceptor interactions carried out so far have often utilized simplified approaches such as adding a set amount of an adrenoceptor agonist to a standard dose of opioid without lowering the opioid dose relative to the opioid-alone group (Park *et al.*, 1996; Förster and Rosenberg, 2004; Paech *et al.*, 2004). It may be of value for future clinical research to approximate more closely what has been done in preclinical studies, without undertaking a full isobolographic analysis. For example, in the opioid–adrenoceptor combination group, dosing could be given in a fixed ratio starting at a fraction of the amount of opioid given in the opioid-alone group; the opioid dose could be chosen based on preclinical studies of the chosen drug combination. This approach may reveal synergism that would otherwise be masked by a high initial opioid dose.

To date, four pairs of receptors capable of mediating synergistic interactions have emerged as potential drug targets for the development of synergy-based therapies: α_{2A}-MOP, α_{2A}-DOP, α_{2C}-MOP and α_{2C}-DOP. Each pair presents advantages and limitations for translation into effective clinical pain treatments.

The benefit of combining morphine or other MOP receptor agonists with clonidine has been demonstrated in clinical studies (Coombs *et al.*, 1986; Park *et al.*, 1996; Siddall *et al.*, 2000; Lena *et al.*, 2003; Förster and Rosenberg, 2004; Paech *et al.*, 2004; for review, see Walker *et al.*, 2002; Gregoretti *et al.*, 2009). In preclinical studies, morphine and clonidine analgesic synergy has been observed in models of acute pain (Ossipov *et al.*, 1990; Roerig *et al.*, 1992; Fairbanks and Wilcox, 1999; Chabot-Doré *et al.*, 2013), a model of neuropathic pain (Ossipov *et al.*, 1997; Fairbanks *et al.*, 2000a), a rodent model of low back pain (Tajerian *et al.*, 2012) and the formalin test (Przesmycki *et al.*, 1997). While MOP receptors and α_{2A}-adrenoceptors mediate the synergistic interaction of this combination in naïve/healthy animals, it is not known if other receptors are involved in these interactions in chronic pain. In models of neuropathic pain, spinal α_2-adrenoceptor agonists ameliorate signs of mechanical allodynia and heat hyperalgesia by activating α_{2A}-adrenoceptors (Kingery *et al.*, 2000; Malmberg *et al.*, 2001). However, the antinociceptive effect of spinally administered clonidine has been shown to switch from α_{2A} in non-injured conditions to α_{2C}-adrenoceptors, after nerve injury (Duflo *et al.*, 2002). This observation is supported by the decreased expression of α_{2A}-adrenoceptors in the superficial lamina of the spinal cord in three rodent models of painful nerve injury, while the expression of α_{2C}-adrenoceptors was maintained (Stone *et al.*, 1999). The therapeutic potential of the α_{2A}-adrenoceptors to relieve neuropathic pain may therefore be limited in certain models. Furthermore, the efficacy of moxonidine, which is thought to act mostly on α_{2C}-adrenoceptors, in neuropathic models underscores the potential clinical utility of these adrenoceptors in the treatment of neuropathic pain (Fairbanks *et al.*, 2000a).

Clinically, patients presenting with neuropathic pain have a reduced sensitivity to morphine and other opioids (Arnér and Meyerson, 1988). Lower spinal morphine potency and efficacy are also observed in rodent models of neuropathic pain and are associated with reduced MOP receptor function and receptor expression (Ossipov *et al.*, 1995). These changes in α_{2A}-adrenoceptors and MOP receptors in chronic pain conditions could explain the discrepant conclusions obtained in clinical studies examining the combination of morphine and clonidine, depending on the pain condition under study (Ackerman *et al.*, 2003).

The synergistic interaction mediated by DOP receptors and α_{2A}-adrenoceptors has not been tested in chronic pain models, so this receptor pair has yet to be validated preclinically for the relief of chronic pain. While there is no clinically approved DOP agonist for the treatment of pain or other conditions, a growing body of preclinical evidence suggests that the DOP receptor represents a promising drug target (Pradhan *et al.*, 2011). The potency of spinally delivered DOP-selective agonists is enhanced in models of neuropathic (Mika *et al.*, 2001; Holdridge and Cahill, 2007) and inflammatory pain (Stewart and Hammond, 1994; Cahill *et al.*, 2003). Unlike morphine, acute and chronic DeltII treatment does not lead to analgesic tolerance (Beaudry *et al.*, 2009). Furthermore, recently developed DOP agonists have reduced side effects at doses effective against hyperalgesia and allodynia, when compared with morphine or SNC80 (Codd *et al.*, 2009; Nozaki *et al.*, 2012). Therefore, the DOP receptor is a promising alternative therapeutic target to MOP receptors for chronic pain conditions and opioid–adrenoceptor drug combinations that activate DOP receptors to produce analgesic synergy may have a better therapeutic profile than MOP-mediated synergistic interactions.

Conclusions and future directions

To better understand opioid–adrenoceptor analgesic synergy, and to exploit synergistic interactions for therapeutic benefit, several key questions need to be answered. First, does synergy also occur in the side effects? If similar synergistic interactions also occur in undesired side effects, then the therapeutic benefits will be limited. While few preclinical studies have directly addressed this question (Tajerian *et al.*, 2012), clinical studies have indicated that side effects remain generally unchanged with drug combinations that enhance analgesia

or decrease overall opioid requirement (Förster and Rosenberg, 2004; Paech *et al.*, 2004). Additional studies are necessary to fully characterize the impact of opioid–adrenoceptor agonist co-administration on analgesia versus undesired side effects.

A second key area of future research is in the potential therapeutic benefits of opioid and adrenoceptor agonist co-administration in chronic pain. To date, the majority of preclinical and clinical studies have focused on acute pain models. A few reports have shown that opioid–adrenoceptor synergy also occurs in rodent models of chronic pain (Ossipov *et al.*, 1997; Fairbanks *et al.*, 2000a; Tajerian *et al.*, 2012). Therefore, the clinical benefits of opioid–adrenoceptor synergy in chronic pain conditions warrant further investigation.

As the treatment of chronic pain involves the long-term use of analgesic drugs, the development of tolerance to opioid–adrenoceptor combinations should also be evaluated. Whereas one preclinical study has shown that synergy between clonidine and morphine is maintained in mice that have developed tolerance to morphine (Fairbanks and Wilcox, 1999), it is not clear if chronic combination therapy is protective against the development of tolerance. In a pilot study, patients with chronic low back and leg pain receiving a polyanalgesic combination such as morphine and clonidine via an i.t. drug delivery system reported a good to excellent treatment efficacy after a 24 month period (Rainov *et al.*, 2001), although moderate dose escalation of morphine was observed over the trial period. Therefore, the effects of chronic co-administration of the opioid–adrenoceptor agonist pair itself – will synergy become additive or sub-additive? will tolerance develop? – has not yet been thoroughly explored.

Finally, understanding the molecular and cellular mechanisms underlying opioid–adrenoceptor synergy will open new avenues for drug development, notably by identifying opioid–adrenoceptor heteromers as drug targets. Advances in GPCR research could accelerate these discoveries as we are only beginning to understand how heteromer formation may affect GPCR function. As information becomes available for heteromeric receptors of interest, it may be possible to design molecules that selectively and effectively target heteromers rather than monomers, potentially manifesting synergistic properties. For example, high throughput screening of a molecular library recently identified a small molecule, CYM51010, with selectivity for MOP-DOP heterodimers over MOP and DOP monomers (Gomes *et al.*, 2013); analogous discoveries may be made for α_2-adrenoceptor-opioid receptor heteromers.

In summary, preclinical and clinical studies have demonstrated the potential for drug combination therapies to increase effectiveness of pain management. Given that some of the agonists that were demonstrated to interact synergistically are already used clinically, significant preclinical advances could lead to a rapid translation to patients and a broader use of opioid–adrenoceptor combination therapies. In addition, determination of the mechanisms underlying interactions between various opioid and adrenoceptor agonists may provide additional targets and insights for better exploitation of signalling pathways capable of producing enhanced analgesia. This information would be of benefit for determining the type of clinical conditions where combination therapies may be most useful, as well as which combinations may be best suited for a given therapeutic goal.

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Conflict of interest

None.

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