Themed Section: Opioids: New Pathways to Functional Selectivity

REVIEW

Analgesic synergy between opioid and α₂-adrenoceptors

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Opioid and α₂-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 α₂-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 α₂-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 α₂-adrenoceptor agonists and that α₂-adrenoceptor agonists can mediate synergy through either the α₂A or the α₂C 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-Ala², NMe-Phe⁴, Gly-ol⁵]-enkephalin; DeltII, [D-Ala²]-deltorphin II; DOP, δ opioid; DPDPE, [D-Pen², D-Pen⁵]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-l-piperazinyl]-3-methoxybenzyl]-N,N-diethyl-benzamide; SP, substance P; ST-91, 2-(2,6-diethylphenylamino)-2-imidazoline HCl; VGCC, voltage-gated Ca²⁺ 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
opioid therapies could be improved upon, and co-delivery of opioids with other analgesics has shown promise for enhanced analgesic utility.

Agonists acting at α2-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 α2-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 α2-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 α2-adrenoceptor subtypes, α2A, α2B and α2C, which are activated by noradrenaline and adrenaline in the CNS and peripheral nervous system (Bylund et al., 1994). Opioid receptors and α2-adrenoceptors are preferentially coupled to the Pertussis toxin (PTX)-sensitive Gαi/o subunit. Gαi/o inhibits the production of cAMP by adenylyl 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 Ca2+ 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 α2-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 isobolo-graphic 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 ED50 response is calculated based on the single drug dose–response curves and statistically compared with the experimental ED50 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-Pen2, D-Pen2enkephalin (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 α2-adrenoceptor agonist potentiated morphine antinociception, and vice versa (Hylden and Wilcox, 1983; Wilcox et al., 1987), suggesting that opioid and α2-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 α2-adrenoceptor agonists in animal models of nociception

<table>
<thead>
<tr>
<th>Opioid agonist</th>
<th>Adrenoceptor agonist</th>
<th>Route</th>
<th>Species</th>
<th>Model*</th>
<th>Behavioural assay</th>
<th>Interaction</th>
<th>Notes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>Clonidine</td>
<td>i.t.</td>
<td>Rat</td>
<td>Naive</td>
<td>TF</td>
<td>Synergistic</td>
<td></td>
<td>Ossipov et al., 1990</td>
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<tr>
<td></td>
<td></td>
<td>i.v.</td>
<td></td>
<td></td>
<td>HP</td>
<td>Additive</td>
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<td></td>
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<td></td>
<td></td>
<td>TF</td>
<td>Additive</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>Naive</td>
<td>SP, TF</td>
<td>Synergistic</td>
<td></td>
<td>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</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.t.</td>
<td>Rat, mouse</td>
<td>SNL</td>
<td>TF, MS</td>
<td>Synergistic</td>
<td></td>
<td>Ossipov et al., 1997; Fairbanks et al., 2000a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.t.</td>
<td>Rat</td>
<td>Formalin assay</td>
<td>FT</td>
<td>Synergistic</td>
<td>Synergy observed in formalin pain model.</td>
<td>Przesmycki et al., 1997</td>
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<tr>
<td></td>
<td></td>
<td>i.p.</td>
<td>Mouse</td>
<td>SPARC-null</td>
<td>TS</td>
<td>Synergistic</td>
<td>Synergy ameliorates signs of axial pain in a mouse model of low back pain.</td>
<td>Tajerian et al., 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>Morphine tolerant</td>
<td>TF</td>
<td>Additive or Synergistic</td>
<td>Interaction depends on the morphine tolerance and drug combination paradigms used.</td>
<td>Roerig, 1995; Fairbanks and Wilcox, 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>PTX</td>
<td>TF</td>
<td>Synergistic</td>
<td></td>
<td>Roerig and Howse, 1996; Wei et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>ω-agatoxin IVA</td>
<td>TF</td>
<td>Additive</td>
<td>Synergy involves P-type VGCC.</td>
<td>Roerig and Howse, 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>PTX + ω-conotoxin</td>
<td>TF</td>
<td>Additive</td>
<td>Morphine–clonidine synergy requires both G protein and N-type VGCC activation.</td>
<td>Wei et al., 1996</td>
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<tr>
<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>Chelerythrine, Calphostin C</td>
<td>TF</td>
<td>Additive</td>
<td>PKC, but not PKA, inhibition blocks morphine clonidine synergy.</td>
<td>Wei and Roerig, 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>H-89</td>
<td>TF</td>
<td>Synergistic</td>
<td></td>
<td>Schuster et al., 2013</td>
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<tr>
<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>PKCe-KO</td>
<td>TF</td>
<td>Synergistic</td>
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<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>DOP-KO</td>
<td>SP</td>
<td>Synergistic</td>
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<tr>
<td>Substance</td>
<td>Species</td>
<td>Naive</td>
<td>SP/MS</td>
<td>Effect</td>
<td>Reference</td>
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<td>Noradrenaline</td>
<td>Mouse</td>
<td>Naive</td>
<td>SP</td>
<td>Synergistic</td>
<td>Morphine + noradrenaline interact synergistically.</td>
<td>Roerig et al., 1992</td>
<td></td>
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<tr>
<td>ST-91</td>
<td>Rat</td>
<td>Naive</td>
<td>HP</td>
<td>Synergistic</td>
<td>Morphine + ST-91 interact synergistically.</td>
<td>Monasky et al., 1990</td>
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</tr>
<tr>
<td>Moxonidine</td>
<td>Mouse</td>
<td>Naive</td>
<td>SP</td>
<td>Synergistic</td>
<td>Morphine + moxonidine interaction is synergistic in both naive and neuropathic pain model.</td>
<td>Fairbanks et al., 2000a, b</td>
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<tr>
<td>Brimonidine</td>
<td>Mouse</td>
<td>WT, PKCc-KO</td>
<td>TF</td>
<td>Synergistic</td>
<td>PKCc is not necessary for morphine-brimonidine interaction.</td>
<td>Schuster et al., 2013</td>
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<tr>
<td>DAMGO</td>
<td>Clonidine</td>
<td>Mouse</td>
<td>Naive</td>
<td>TF</td>
<td>Synergistic or additive</td>
<td>DAMGO + clonidine interaction is assay-dependent.</td>
<td>Roerig, 1995</td>
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<td>Roerig et al., 1992; Chabot-Doré et al., 2013</td>
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<tr>
<td>Noradrenaline</td>
<td>Mouse</td>
<td>Naive</td>
<td>SP</td>
<td>Antagonistic</td>
<td>DAMGO + noradrenaline interaction is sub-additive</td>
<td>Roerig et al., 1992</td>
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<tr>
<td>Moxonidine</td>
<td>Mouse</td>
<td>Naive</td>
<td>SP</td>
<td>Antagonistic</td>
<td>DAMGO + moxonidine interaction is sub-additive</td>
<td>Fairbanks et al., 2000b</td>
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<td>Brimonidine</td>
<td>Mouse</td>
<td>D79N-α2A-adrenoceptor-</td>
<td>SP</td>
<td>Synergistic</td>
<td>DAMGO + brimonidine synergy is mediated through the α2A-adrenoceptor.</td>
<td>Stone et al., 1997</td>
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<td>Endomorphin</td>
<td>Clonidine</td>
<td>Mouse</td>
<td>Naive</td>
<td>TF</td>
<td>Synergistic</td>
<td>PKCc is not required for endomorphin-mediated interactions with clonidine or brimonidine.</td>
<td>Schuster et al., 2013</td>
<td></td>
</tr>
<tr>
<td>Brimonidine</td>
<td>Mouse</td>
<td>PKCc-KO</td>
<td>TF</td>
<td>Synergistic</td>
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<td>Fentanyl</td>
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<td>Naive</td>
<td>TF, HP</td>
<td>Additive</td>
<td>Outcome of interaction depends on route of administration.</td>
<td>Ossipov et al., 1990</td>
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<td>Meperidine</td>
<td>Clonidine</td>
<td>i.v.</td>
<td>Naive</td>
<td>TF, HP</td>
<td>Synergistic</td>
<td>Outcome of interaction depends on route of administration.</td>
<td>Ossipov et al., 1990</td>
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</tbody>
</table>

*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>
<thead>
<tr>
<th>Opioid agonist</th>
<th>Adrenoceptor agonist</th>
<th>Route</th>
<th>Specie</th>
<th>Model*</th>
<th>Behavioural assay</th>
<th>Interaction</th>
<th>Notes</th>
<th>References</th>
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<tr>
<td>DeltII</td>
<td>Clonidine</td>
<td>i.t.</td>
<td>Mouse</td>
<td>Naive</td>
<td>TF, SP</td>
<td>Synergistic</td>
<td>DeltII + clonidine interact synergistically in two nociceptive pain models.</td>
<td>Overland et al., 2009; Schuster et al., 2013; Chabot-Doré et al., 2013</td>
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<tr>
<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>DOP-KO</td>
<td>SP</td>
<td>No interaction</td>
<td>DeltII–clonidine synergy requires DOP.</td>
<td>Chabot-Doré et al., 2013</td>
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<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>Chelerythrine</td>
<td>TF</td>
<td>Additive</td>
<td>DeltII–clonidine synergy is PKCe-dependent.</td>
<td>Overland et al., 2009</td>
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<td></td>
<td>PKCe-KO</td>
<td>Additive</td>
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<td>Schuster et al., 2013</td>
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<td>Chelerythrine</td>
<td>Synergistic</td>
<td>DeltII–moxonidine synergistic interaction is dependent on α2C-, not α2A-adrenoceptors.</td>
<td>Fairbanks et al., 2000b; Fairbanks et al., 2002</td>
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<td>Mouse</td>
<td>Naive</td>
<td>SP</td>
<td>Additive</td>
<td>Schuster et al., 2013</td>
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<td>α2C-adrenoceptor KO</td>
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<td></td>
<td>D79N-α2A-adrenoceptor</td>
<td>Additive</td>
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<tr>
<td>ST-91</td>
<td>i.t. Mouse</td>
<td>WT;</td>
<td>D79N-α2A_α2C-KO</td>
<td>SP</td>
<td>Synergistic</td>
<td>DeltII–ST-91 synergy does not require α2C or α2A-adrenoceptors.</td>
<td>Stone et al., 2007</td>
<td></td>
</tr>
<tr>
<td>Brimonidine</td>
<td>i.t. Mouse</td>
<td>Naive</td>
<td>PKCe-KO, PKCe inhibitor</td>
<td>TF</td>
<td>Synergistic</td>
<td>Inhibiting PKCe/β or PKCe6 does not impair DeltII–brimonidine synergy, but inhibiting or genetically deleting PKCe does.</td>
<td>Schuster et al., 2013</td>
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<td>PKCe/β inhibitor</td>
<td>Additive</td>
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<td></td>
<td>PKCe/δ inhibitor</td>
<td>Synergistic</td>
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<td></td>
<td></td>
<td>i.t.</td>
<td>Mouse</td>
<td>Naive</td>
<td>SP</td>
<td>Synergistic</td>
<td>DeltII–brimonidine synergy requires α2A-adrenoceptors.</td>
<td>Stone et al., 1997</td>
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<td>D79N-α2A-adrenoceptor</td>
<td>Additive</td>
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<td>DPDPE</td>
<td>Clonidine</td>
<td>i.t.</td>
<td>Rat, Mouse</td>
<td>Naive</td>
<td>TF, SP</td>
<td>Synergistic</td>
<td>DPDPE + clonidine interact synergistically in two nociceptive pain models.</td>
<td>Roerig et al., 1992; Ossipov et al., 1990</td>
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<td>Noradrenaline</td>
<td>i.t. Mouse</td>
<td>Naive</td>
<td>SP</td>
<td>Synergistic</td>
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<td>Brimonidine</td>
<td>i.t. Mouse</td>
<td>Naive</td>
<td>MOP-KO</td>
<td>Synergistic</td>
<td>MOP is not necessary for DPDPE–brimonidine interaction</td>
<td>Guo et al., 2003</td>
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<tr>
<td>SNC80</td>
<td>Clonidine</td>
<td>i.t.</td>
<td>Mouse</td>
<td>Naive</td>
<td>TF</td>
<td>Synergistic</td>
<td>PKCe is required for SNC80-mediated synergistic interaction with clonidine or brimonidine.</td>
<td>Schuster et al., 2013</td>
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<td>PKCe-KO</td>
<td>Additive</td>
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*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 α2-adrenoceptors in locus coeruleus 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, α2A and α2C, capable of producing synergetic opioid–adrenoceptor interactions. The MOP- and DOP receptor-mediated interactions with different α2-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 α2-adrenoceptor agonists like clonidine exert a vasoconstrictive effect (Asada and Lee, 1992; lida 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-Ala2, NMe-Phe4, Gly-ol5]-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 α2-adrenoceptor agonist, 2-(2,6-diethylphenylamino)-2-imidazoline HCl (ST-91; Monaskey et al., 1990), morphine and ST-91 produce antinociceptive synergy (Yasuoka and Yaksh, 1983; Monaskey et al., 1990; Nagasaka and Yaksh, 1990). Therefore, it is unlikely that pharmacokinetic effects of α2-adrenoceptor agonists contribute significantly to opioid–adrenoceptor synergy at the spinal level.

**Pharmacodynamic mechanisms**

Synergistic interactions between opioid and α2-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. α2A-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, α2C-adrenoceptors are also expressed on descending noradrenergic axon terminals where they act as autoreceptors to inhibit noradrenaline release (Li et al., 2000; Gilbsbach 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 α2C 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 $\alpha_2$-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 $\alpha_2$-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 greater 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 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 spinal (Hylden and Wilcox, 1982; Tung and Yaksh, 1982) raised the possibility that these drugs could also interact synergistically with $\alpha_2$-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 $\alpha_2$-adrenoceptors has been observed with several different agonist combinations (Table 2). The DOP receptor agonists [D-Ala]$^4$-deltorphin II (DeltII), DPDPPE and (±)-4-[[[(R)-α-[(25S)R]-4-allyl-2,5-dimethyl-1-piperazinyl]-3-methoxybenzyl]-N,N-diethyl-benzamide (SNC80) all produce antinociceptive synergy when co-delivered spinal with the $\alpha_2$-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 $\alpha_2$-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 DPDPPE 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 $\alpha_2$-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.

$\alpha_2$-adrenoceptor subtypes mediating synergistic interaction with opioids

Because of the general lack of subtype selectivity of $\alpha_2$-adrenoceptor agonists and antagonists, the identification of $\alpha_2$-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 $\alpha_2A$-adrenoceptor, the $D79N$-$\alpha_2A$ receptor, provided evidence that this adrenoceptor subtype mediated the antinociceptive, hypotensive and sedative effects of $\alpha_2$-adrenoceptor agonists (MacMillan et al., 1996; Lakhiani et al., 1997). Three opioid–adrenoceptor combinations were then tested in these mice to determine the role of $\alpha_2A$-adrenoceptors in opioid–adrenoceptor synergy. For brimonidine and DeltII or DAMGO, the synergistic interaction was mediated by $\alpha_2A$-adrenoceptors (Stone et al., 1997), as mice expressing the dysfunctional $\alpha_2A$-adrenoceptors exhibited no analgesic synergy. In contrast, the $\alpha_2C$-adrenoceptor was not necessary for the synergistic interaction between moxonidine and DeltII, which was instead mediated by the $\alpha_2C$-adrenoceptor (Fairbanks et al., 2002). Another $\alpha_2$-adrenoceptor agonist, ST-91, interacts synergistically with DeltII in mice with the mutant $D79N$-$\alpha_2A$-adrenoceptor or in $\alpha_2A$-adrenoceptor-KO mice (Stone et al., 2007). This outcome suggests that either ST-91 is versatile and can produce synergy via either $\alpha_2A$ or $\alpha_2C$-adrenoceptors, or that the $\alpha_2B$ receptors mediate this interaction. Thus, depending on the adrenoceptor agonist used, it appears that either $\alpha_2A$ or $\alpha_2C$ 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 $G_{\alphaq}$ 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 $G_{\alphaq}$, 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 Gαq 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.

**PKCe 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 DeltIII-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 DeltIII, clonidine and their combination was lost when PLC was inhibited. This result suggests that PLC contributes to the antinociceptive effect of DeltIII 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 α2A-adrenoceptor agonists. Work by Schuster et al. (2013) using isoform-specific peptide inhibitors and the ε isoform of PKC (PKCe)-knockout mice has shown that interactions involving α2A-adrenoceptors and DOP receptors specifically require PKCe. PLCε is directly upstream of PKCe in primary afferent neurons (Joseph et al., 2007), and Gαε-associated Gβγ and Gαq subunits can synergistically activate PLCβ1 (Philip et al., 2010; Rebes et al., 2011). Considering this, activation of either α2A adrenoceptors or DOP receptors alone might be insufficient to induce activation of PKCe. Instead, co-activation of different Gα subunits by α2A-adrenoceptors and DOP receptors might be required for signalling to PKCe. Support for this hypothesis comes from the finding that activation or inhibition of PKCe 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 PKCe leading to a subsequent increase in potency of the other agonist, and suggests instead that receptor co-activation is necessary for involvement of PKCe in enhanced analgesia.

It is not clear how PKCe is involved in the enhanced analgesia produced upon co-delivery of an α2A-adrenoceptor agonist with a DOP receptor agonist. There is evidence that PKCe 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 α2A-adrenoceptors and DOP receptors, it is possible that PKCe may alter surface availability of these receptors via similar mechanisms. PKCe may also play a role in induction of functional competence of DOP receptors by certain stimuli. In primary afferent neurons, bradykinin may 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 α2A-adrenoceptors can both exert their analgesic action by inhibiting transmitter release from primary afferent terminals (Glau et al., 1994; Kawasaki et al., 2003). Evidence suggests that the synergistic interaction between DeltIII and clonidine is mediated at the level of the primary afferent nerve terminal; for example, this drug combination synergistically inhibited KCI-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 α2A-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 α2A-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 heterooligomers that mediate opioid–adrenoceptor synergy in vivo. In HEK cells co-expressing epitope-tagged DOP receptors and α2A-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 α2A-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-α2A-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 α2A-adrenoceptor recruits distinct downstream signalling effectors resulting in the activation of PKCe, which would in turn mediate the analgesic synergy.

**Molecular mechanisms underlying MOP receptor-mediated synergistic interactions**

As interactions between MOP receptors and α2-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 α2-adrenoceptors with MOR 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 Gαi/o, signalling pathway usually associated with the activation of opioid and adrenoceptors. As uncoupling the Gαi/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 PKCe-knockout mice indicate that this isoform mediates neither spinal morphine analgesia nor interactions between morphine and α2A-adrenoceptor agonists (Schuster et al., 2013), but involvement of other isoforms has not been evaluated.

MOR receptors and α2A-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 MOR receptors (Wang et al., 2010) and α2A-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 MOR receptors and for α2A-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 MOR receptors and α2A-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 MOR receptors and α2A-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 MOR receptor and α2A-adrenoceptors in nociceptors. Thus, MOR-mediated synergistic interactions with α2A-adrenoceptor agonists may result from interactions between MOR receptors and α2A-adrenoceptors within the same cells, which could be mediated by the heteromerization of these receptors.

In heterologous expression systems, MOR receptors and α2A-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 MOR receptors and α2A-adrenoceptors has important functional consequences. Vilardaga et al. (2008) studied the effect of ligand-induced cross-conformational change between MOR receptors and α2A-adrenoceptors and how it modulates coupling to downstream signalling. They showed that morphine reduced noradrenaline-mediated conformational changes in the α2A-adrenoceptors and downstream activation of Gαi/o induced by noradrenaline. Furthermore, the inhibition of Gαi/o signalling was strongly correlated with the inhibition of the ERK1/2 signalling pathway, suggesting that the activation state of MOR receptors influences the output of the α2A-adrenoceptors. This study hence demonstrated the ability of MOR receptors to allosterically modulate α2A-adrenoceptors, but did not test the reverse, that is the ability of α2A-adrenoceptors to allosterically modulate MOR receptors, which does not exclude the possibility that allosteric modulations work symmetrically within this receptor pair. In the presence of a single agonist, MOR receptors and α2A-adrenoceptors normally activate MAPK signalling pathways. Interestingly, the observation that Gαi/o 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 MOR receptors and α2A-adrenoceptors (Jordan et al., 2003). Therefore, the suppression of Gαi/o protein coupling and downstream signalling may indicate that MOR-α2A-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, MOR-α2A-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 α2-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 \textit{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 \textit{et al}, 1996; Förster and Rosenberg, 2004; Paech \textit{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: $\alpha_2A$-MOP, $\alpha_2A$-DOP, $\alpha_2C$-MOP and $\alpha_2C$-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 \textit{et al}, 1986; Park \textit{et al}, 1996; Siddall \textit{et al}, 2000; Lena \textit{et al}, 2003; Förster and Rosenberg, 2004; Paech \textit{et al}, 2004; for review, see Walker \textit{et al}, 2002; Gregoretti \textit{et al}, 2009). In preclinical studies, morphine and clonidine analgesic synergy has been observed in models of acute pain (Ossipov \textit{et al}, 1990; Roerig \textit{et al}, 1992; Fairbanks and Wilcox, 1999; Chabot-Doré \textit{et al}, 2013), a model of neuropathic pain (Ossipov \textit{et al}, 1997; Fairbanks \textit{et al}, 2000a), a rodent model of low back pain (Tajerian \textit{et al}, 2012) and the formalin test (Przesmycki \textit{et al}, 1997). While MOP receptors and $\alpha_2A$-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 $\alpha_2A$-adrenoceptor agonists ameliorate signs of mechanical allodynia and heat hyperalgesia by activating $\alpha_2A$-adrenoceptors (Kingery \textit{et al}, 2000; Malmberg \textit{et al}, 2001). However, the antinociceptive effect of spinaly administered clonidine has been shown to switch from $\alpha_2A$ in non-injured conditions to $\alpha_2C$-adrenoceptors, after nerve injury (Duflo \textit{et al}, 2002). This observation is supported by the decreased expression of $\alpha_2A$-adrenoceptors in the superficial lamina of the spinal cord in three rodent models of painful nerve injury, while the expression of $\alpha_2C$-adrenoceptors was maintained (Stone \textit{et al}, 1999). The therapeutic potential of the $\alpha_2A$-adrenoceptors to relieve neuropathic pain may therefore be limited in certain models. Furthermore, the efficacy of moxonidine, which is thought to act mostly on $\alpha_2C$-adrenoceptors, in neuropathic models underscores the potential clinical utility of these adrenoceptors in the treatment of neuropathic pain (Fairbanks \textit{et al}, 2000).

Clinically, patients presenting with neuropathic pain have a reduced sensitivity to morphine and other opioids (Arner 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 \textit{et al}, 1995). These changes in $\alpha_2C$-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 \textit{et al}, 2003).

The synergistic interaction mediated by DOP receptors and $\alpha_2A$-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 \textit{et al}, 2011). The potency of spinally delivered DOP-selective agonists is enhanced in models of neuropathic (Mika \textit{et al}, 2001; Holdridge and Cahill, 2007) and inflammatory pain (Stewart and Hammond, 1994; Cahill \textit{et al}, 2003). Unlike morphine, acute and chronic DelTII treatment does not lead to analgesic tolerance (Beaudry \textit{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 \textit{et al}, 2009; Nozaki \textit{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 \textit{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 α2-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.

References


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