Published in final edited form as:

Pharmacol Ther. 2009 August ; 123(2): 224–238. doi:10.1016/j.pharmthera.2009.04.001.

Pharmacological Profiles of Alpha 2 Adrenergic Receptor Agonists Identified Using Genetically Altered Mice and Isobolographic Analysis

Carolyn A. Fairbanks^{1,3,4}, Laura S. Stone², and George L. Wilcox^{3,4,5}

¹Department of Pharmaceutics, College of Pharmacy, University of Minnesota, Minneapolis

²Centre for Research on Pain, McGill University, Montreal QE Canada

³Department of Neuroscience, Medical School, University of Minnesota, Minneapolis MN 55455

⁴Department of Pharmacology, Medical School, University of Minnesota, Minneapolis MN 55455

⁵Department of Dermatology, Medical School, University of Minnesota, Minneapolis MN 55455

Abstract

Endogenous, descending noradrenergic fibers convey powerful analgesic control over spinal afferent circuitry mediating the rostral transmission of pain signals. These fibers target alpha 2 adrenergic receptors (α_2 ARs) on both primary afferent terminals and secondary neurons, and their activation mediates substantial inhibitory control over this transmission, rivaling that of opioid receptors which share similar a similar pattern of distribution. The terminals of primary afferent nociceptive neurons and secondary spinal dorsal horn neurons express α_{2A} AR and α_{2C} AR subtypes, respectively. Spinal delivery of these agents serves to reduce their side effects, which are mediated largely at supraspinal sites, by concentrating the drugs at the spinal level. Targeting these spinal α_2 ARs with one of five selective therapeutic agonists, clonidine, dexmedetomidine, brimonidine, ST91 and moxonidine, produces significant antinociception that can work in concert with opioid agonists to yield synergistic antinociception. Application of several genetically altered mouse lines had facilitated identification of the primary receptor subtypes that likely mediate the antinociceptive effects of these agents. This review provides first an anatomical description of the localization of the three subtypes in the central nervous system, second a detailed account of the pharmacological history of each of these six primary agonists, and finally a comprehensive report of the specific interactions of other GPCR agonists with each of the six principal α_2 AR agonists featured.

Keywords

Alpha-2 adrenergic receptor; interactions; opioid receptor; antinociception; analgesia; receptor subtype

© 2009 Elsevier Inc. All rights reserved.

Corresponding Author:, Dr. George L. Wilcox, 6-145 Jackson Hall, 321 Church St. SE, University of Minnesota, Minneapolis MN 55455, Phone: +1-612-625-1474, Fax: +1-612-626-5009, email: george@umn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1.0 General Introduction

Adrenergic receptors mediate the physiological actions of the catecholamine neurohormones, epinephrine and norepinephrine (NE). All or most of the latter neurotransmitter derives from eight of the thirteen catecholaminergic brainstem nuclei that project to the entire central nervous system. The catecholamines are synthesized from the amino acid L-tryptophan, which is first converted to L-3,4 dihydroxy phenylalanine (L-DOPA) by tyrosine hydroxylase (TH), to dopamine (DA) by DOPA decarboxylase, to NE by dopamine β -hydroxylase (D β H) and finally to epinephrine by phenylethanolamine N-methyltransferase (PNMT). Pharmacologically subdivided into α_1 (α_{1A} AR, α_{1B} AR, α_{1C} AR), α_2 (α_{2A} AR, α_{2B} AR, α_{2C} AR) and beta (β_1 AR, β_2 AR, β_3 AR) adrenergic receptor subtypes, they belong to the group A rhodopsin-like G protein-coupled receptor class. This review focuses on the localization, function and pharmacology of the three subtypes of α_2 AR, α_{2A} , α_{2B} , and α_{2C} (for review see (Aantaa et al., 1995; MacDonald et al., 1997; Philipp et al., 2002)), particularly with regard to spinal analgesic actions and interactions with opioids.

The endogenous noradrenergic system imposes powerful inhibitory effects on the spinal cord dorsal horn circuitry that processes afferent nociceptive input from the periphery. This endogenous analgesic influence originates largely, but not exclusively, from the brainstem locus coeruleus (A6 nucleus; (Nicholas et al., 1996)) which mediates responses to stress and fear. That part of this action is imposed presynaptically at the level of the spinal cord is attested to by structural (Stone et al., 1998) and functional (Kawasaki et al., 2003; Sonohata et al., 2004) observations. That part of this action is imposed postsynaptically is attested to by the observation that NE or other α_2 AR agonists can hyperpolarize secondary dorsal horn neurons *in vivo* (Sonohata et al., 2004). This dual localization of inhibitory receptors activated by a common endogenous agonist affords descending noradrenergic fibers highly effective control of incoming nociceptive impulses.

The fact that opioid agonists also act to activate descending monoaminergic inhibitory tracts perhaps explains the potency of opioid agonists delivered systemically (Bouaziz et al., 1996). In other words, systemically delivered opioids almost certainly invoke auto-synergistic site-to-site interactions that contribute importantly to their emergent analgesic effects (Bodnar, 2000). α_2 AR-selective agonists have been known for decades to have analgesic and anesthetic properties. Clinical application of these agonists, alone and as opioid co-adjuvants, remains an active area of development; they are particularly useful as adjuvants for pain management and as anesthetic-sparing agents (Sanders & Maze, 2007). Clonidine is approved for spinal use and may be applied intrathecally in patients who have become tolerant to intrathecal opioids, particularly morphine; clonidine provides analgesia in these patients while the patients' tolerance is reduced over time. Less commonly, clonidine is used as a co-adjuvant with morphine, both being administered intrathecally. Additionally, a recent case report features an instance where intrathecally administered dexmedetomidine restored morphine analgesia in a morphine tolerant patient. (Ugur et al., 2007). Dexmedetomidine is, however, not widely delivered intrathecally since the formulation has not undergone the rigorous toxicity evaluation required for direct central delivery.

While there has been extensive description of the use of α_2 AR-selective and opioid agonists delivered as monotherapeutics and to some degree the synergism of their combined use, there has been minimal focus upon their respective receptor subtype activation both as monotherapeutics and combinations. The introduction of genetically altered mice in the 1990s provided a unique opportunity to evaluate the receptor subtype requirements of a broad spectrum of commonly used α_2 AR-selective agonists both as monotherapeutics and as combinations with a variety of opioids. The outcomes of the last ten years of studies illustrate several key points. First, route of administration greatly impacts the participation of various

receptor subtypes in the analgesic effect (or lack of effect) of a drug or drug combination. Second, binding affinities *in vitro* do not correlate with receptor requirements *in vivo*. Third, the pharmacological profile of a single agonist does not necessarily represent the pharmacological profile of a class of compounds, even if that agonist has been considered to be the gold standard. Fourth, the performance of two drugs given separately does not necessarily predict the performance of the two drugs given in combination. These four points will be illustrated through this review of the anatomical localization of the various α_2 AR subtypes and the pharmacological profile of α_2 AR-agonists as monotherapeutics and combination adjuvants in normal and mutant mice.

2.0 Anatomical distribution of α_2 -adrenergic receptors

2.1 Distribution of α_2 -adrenergic receptors in pain pathways

α_2 ARs are widely distributed throughout the peripheral and central nervous system (CNS). Agonists acting at α_2 ARs have analgesic properties following both supraspinal (Guo et al., 1996), spinal (Reddy & Yaksh, 1980; Reddy et al., 1980; Yaksh & Reddy, 1981), peripheral (Davis et al., 1991; Dogrul & Uzbay, 2004) and systemic (Paalzow, 1974) administration. This section will focus on the components of noradrenergic pontine nuclei that project caudally to the spinal cord dorsal horn and the spinal targets of these projections (α_2 ARs). Both endogenous NE and the majority of exogenous receptor-selective agonists exert their analgesic effects on these dorsal horn targets; this review will focus on the effects of agonist activation of those α_2 ARs expressed by primary afferent terminals and secondary neurons.

2.2 Descending adrenergic modulation

α_2 ARs play a critical role in endogenous descending pain modulatory systems. The activation of descending fibers results in antinociception at the level of the spinal cord. Yaksh and colleagues first demonstrated in 1979 that analgesia evoked by morphine administration into the periaqueductal grey (PAG) is attenuated by spinal administration of α_2 AR antagonists (Yaksh, 1979). More recently, it was shown that mice lacking spinal NE, either by deletion of the gene coding for dopamine beta-hydroxylase (D β H) or following neurotoxic lesion of D β H-expressing cells, display signs of chronic hyperalgesia and decreased morphine efficacy (Jasmin et al., 2003; Jasmin et al., 2002). Descending NE fibers have therefore been clearly implicated in both the tonic, endogenous inhibition of nociception and opioid analgesia. More recently, Maze and coworkers demonstrated that nitrous oxide's antinociceptive effects were attributable to activation of noradrenergic neurons in brainstem nuclei (Sawamura et al., 2000).

Extensive evidence supports the expression of α_2 ARs on the cell bodies of descending NE neurons. For example, early autoradiographic studies reported α_2 AR binding in the rostroventromedial medulla (RVM) and locus coeruleus (LC) (Unnerstall et al., 1984). Following the cloning of the α_{2A} , α_{2B} and α_{2C} receptor subtypes, these studies have been extended by examination of α_2 AR subtype mRNA and protein expression by *in situ* hybridization and immunohistochemistry, respectively. Such evidence will be summarized in the following sections on each α_2 AR subtype.

α_{2A} AR—Expression of mRNA encoding α_{2A} AR has been reported in numerous nuclei related to descending modulation of nociception including the LC, periaqueductal gray (PAG), the A4, A5 and A7 cell groups, pontine nuclei and dorsal raphe nuclei in rats (Nicholas et al., 1993; Scheinin et al., 1994) and mice (Wang et al., 1996). Results from immunohistochemical studies are in good agreement with both autoradiographic and mRNA data. Immunoreactivity (-ir) for α_{2A} AR is found in virtually all cells within the LC (Aoki et al., 1994; Rosin et al., 1993; Talley et al., 1996), all of which are also TH-ir (Rosin et al., 1993). Double-staining for

α_{2A} AR and TH is also found in ventrolateral pons and 95% of catecholaminergic cells in the A1, A2, and A5 nuclei express α_{2A} AR, suggesting that the α_{2A} AR is the prominent NE autoreceptor in these neurons (Guyenet et al., 1994). Interestingly, several independent reports describe α_{2A} AR-ir labeling as consisting of both diffuse labeling and sharp, densely labeled puncta, suggesting vesicular localization (Aoki et al., 1994; Talley et al., 1996). The predominant role of α_{2A} ARs is further supported by the nearly complete absence of clonidine and dexmedetomidine inhibition in LC neurons isolated from mice lacking functional α_{2A} ARs. (Lakhlani et al., 1997).

α_{2B} AR—The expression of α_{2B} AR mRNA in the brain is limited to low to moderate expression in the thalamus and has not been detected in descending modulatory pathways in the brain (Nicholas et al., 1993; Scheinin et al., 1994; Wang et al., 1996). Extensive immunohistochemical studies of α_{2B} AR protein expression in brain have not been reported.

α_{2C} AR—Studies examining α_{2C} AR mRNA expression consistently report that this subtype is not expressed in the locus coeruleus. Similarly, other nuclei relevant to descending inhibition such as the PAG or pontine nuclei were either negative or expressed the receptor at very low levels (Nicholas et al., 1993; Scheinin et al., 1994; Wang et al., 1996). In contrast, studies using an α_{2C} AR antibody revealed extensive expression of α_{2C} AR in the brain, including in regions involved in pain modulation (i.e. RVM) as well as in nearly every cell in the LC (Rosin et al., 1996). Whether the discrepancy between these studies is due to cross-reactivity of the antibody to non- α_{2C} AR targets or false negatives in the α_{2C} AR mRNA studies is unknown. However, the inability of clonidine and dexmedetomidine to inhibit LC neurons in mice deficient in functional α_{2A} ARs (Lakhlani et al., 1997) strongly suggests that the α_{2C} ARs are not functionally relevant as autoreceptors on LC neurons.

2.3 Spinal targets of descending modulatory input

Autoradiographic studies using α_2 AR demonstrate that the majority of α_2 ARs in the spinal cord are concentrated in the superficial dorsal horn (Seybold & Elde, 1984; Unnerstall et al., 1984). It is in this region of the spinal cord that the central processes of primary afferent neurons terminate. These receptors may be localized to (1) the terminals of descending fibers, (2) local spinal neurons or (3) the central terminals of primary afferent fibers. The evidence supporting each of these possible localizations is discussed below.

2.3.1 α_2 AR expression on descending NE terminals—Given the extensive expression of α_2 ARs in general and α_{2A} ARs in particular by descending NE neurons, it is reasonable to expect a proportion of spinal α_2 ARs will be localized on descending terminals. This is supported by observations that the pattern of α_2 AR ligand binding, such as for the antagonist [3 H]-rauwolscine, in spinal cord is reminiscent of that observed for D β H-ir, (Roudet et al., 1994). However, direct demonstration of this localization by anatomical methods has been elusive.

Autoradiographic studies have reported that neither cervical spinal cord transection nor neurotoxic lesion of NE fibers by 6-hydroxy DA (6-OHDA) results in a decrease in α_2 AR binding sites in the spinal cord, suggesting that descending terminals do not contribute to spinal α_2 AR content (Howe, et al., 1987a; Roudet et al., 1994). Rather, one such study reported an increase in spinal α_2 AR binding, suggesting an up-regulation of receptors in response to the absence of agonist from descending sources (Roudet et al., 1994). The absence of α_2 ARs on descending NE terminals is further supported by immunohistochemical studies in which neither α_{2A} AR-ir nor α_{2C} AR-ir co-localizes with immunoreactivity for the synthetic enzymes TH, D β H or PNMT (Olave & Maxwell, 2002; Rosin et al., 1993; Stone et al., 1998).

In contrast to the anatomical studies, some functional evidence exists for α_2 AR expression on descending NE terminals (the spinal cord itself lacks intrinsic noradrenergic neurons). In a preparation of nerve terminals (synaptosomes) isolated from spinal cord, the α_2 AR agonist clonidine was found to inhibit NE release (Li et al., 2000). Furthermore, reduction of α_{2A} AR expression with antisense oligonucleotides attenuated the inhibition of NE release by clonidine. These data suggest the presence of a descending autoreceptor at levels too low to be detected by autoradiography or immunohistochemistry. The identification of these autoreceptors as belonging to the α_{2a} AR subtype is consistent with the abundance of α_{2A} AR expression in descending noradrenergic nuclei.

2.3.2 α_2 AR expression by spinal cord neurons—Direct hyperpolarization of neurons in the spinal cord by α_2 AR agonists in spinal cord slice preparations (North & Yoshimura, 1984) and from *in vivo* patch recording (Sonohata et al., 2004) provide strong evidence that α_2 ARs are expressed by a subset of spinal cord neurons. Data demonstrating that local endogenous release of norepinephrine in the spinal cord following PAG stimulation inhibits the responsiveness of dorsal horn neurons suggests that these receptors are targeted by descending circuits (Budai et al., 1998). While there is strong agreement across functional studies that α_2 ARs are expressed on spinal cord neurons, uncertainty surrounds the relative contributions of each subtype.

α_{2a} AR: Studies examining expression of α_{2A} AR mRNA in spinal cord neurons found labeling in the intermediolateral cell column in the thoracic cord as well as throughout the dorsal horn, including in superficial layers (Nicholas et al., 1993; Shi et al., 1999). In contrast, immunohistochemical studies with an α_{2A} AR antibody derived from the third intracellular loop of the receptor identified few scattered cells in deep dorsal and ventral horn but no labeling in the superficial dorsal horn (Rosin et al., 1993; Rosin et al., 1996). An antibody directed against the carboxy terminal of the receptor revealed dense α_{2A} AR-ir in the superficial dorsal horn but, rather than being expressed by local spinal neurons, these receptors were on the terminals of primary afferent fibers (Stone et al., 1998). As a result of the mismatch between the mRNA and immunohistochemical data, it is currently not clear from the anatomical data if the α_{2A} AR subtype is expressed by spinal cord neurons.

Several sets of functional data, however, have emerged recently suggesting that the α_{2A} AR may be expressed by spinal cord neurons. First, a pharmacological study of the action of dexmedetomidine-evoked hyperpolarization of superficial dorsal horn neurons in spinal cord slices suggested that both α_{2A} AR and α_{2C} AR receptors were involved (Ishii et al., 2008). Second, α_2 AR agonist-mediated inhibition of formalin-evoked nocifensive behaviors, shown pharmacologically to be mediated by α_{2A} AR, were not accompanied by an inhibition of substance P (SP) release in the spinal cord, suggesting that the site of action is downstream of the primary afferent terminal (Nazarian et al., 2008). However, given that the selectivity of the currently available antagonists used in these studies is 100-fold or less across the α_2 AR subtypes (Wikberg-Matsson et al., 1995; Wild et al., 1994), assignment of subtype based exclusively on pharmacological data must be viewed with some degree of caution.

α_{2B} AR: While α_{2B} AR mRNA was initially not detected in the spinal cord (Nicholas et al., 1993), later studies identified labeled cells in the superficial dorsal horn of adult rats (Nicholson et al., 2005; Shi et al., 1999). The detection of α_{2B} AR mRNA and protein in embryonic (E14) spinal cord neurons suggests that this receptor may have an important role during development (Huang et al., 2002). Based on the lack of effect of functional knock-out of α_{2a} AR on N2O-induced antinociception, Maze and co-workers concluded that either α_{2B} or α_{2C} ARs mediate this action (Guo et al., 1999).

α_2 CAR: Inconsistencies exist between the mRNA and immunohistochemical (IHC) studies regarding α_2 CAR expression in the superficial dorsal horn. Whereas strong mRNA labeling for α_2 CAR is detected in large cells in the ventral horn, only very weak, diffuse signal was observed in the dorsal horn (Nicholas et al., 1993) (Shi et al., 1999). In contrast, numerous IHC studies have reported α_2 CAR-ir in spinal cord neurons (Olave & Maxwell, 2002; Olave & Maxwell, 2003a,b; Stone et al., 1998). The attenuation of spinal α_2 CAR-ir following treatment with antisense oligonucleotides targeting the α_2 CAR gene supports the specificity of the antisera used in the aforementioned studies (Fairbanks et al., 2002).

The distribution of α_2 CAR-ir in the spinal cord has interesting functional implications. Electron microscopy has revealed that α_2 CAR-ir profiles are composed primarily of axon terminals forming axodendritic synapses (Olave & Maxwell, 2002). It has been shown by confocal microscopy that most (84%) α_2 CAR-ir terminals in the superficial dorsal horn are glutamatergic and originate principally from spinal interneurons (Olave & Maxwell, 2003). Approximately 25% of them also express the endogenous opioid met-enkephalin but little to no expression is observed on glycinergic or cholinergic neurons (Olave & Maxwell, 2002). A study reporting that α_2 AR agonists inhibit endogenous opioid release from spinal cord slices with a pharmacological profile consistent with α_2 CAR (Chen et al., 2008) supports the co-localization of α_2 CAR-ir and enkephalin-ir and suggests a possible mechanism for adrenergic-opioid interactions.

Since most α_2 CAR-ir terminals release excitatory neurotransmitters, activation of α_2 CAR would be expected to inhibit neurotransmission in the spinal cord. When combined with a retrograde tracer to identify spinal projection neurons, α_2 CAR-ir terminals were found to form direct synaptic connections with spinomedullary projection neurons (Olave & Maxwell, 2003). The functional significance of these findings is that α_2 CAR activation likely attenuates the transmission of nociceptive input to supraspinal structures by inhibiting excitatory input onto spinal projection neurons.

In summary, while some disagreement exists in the literature regarding the relative expression of each α_2 AR subtype in the spinal cord, α_2 ARs are present on spinal cord neurons where they likely play a critical role in the modulation of nociceptive input by both endogenous and exogenously administered α_2 AR agonists.

2.3.3 α_2 AR expression by primary afferent neurons—The presence of α_2 ARs on the central terminals of primary afferent fibers was initially demonstrated by Howe et al., (1987b) who showed that α_2 AR binding in the dorsal horn was reduced by ipsilateral ganglionectomies. The functional implication of this expression pattern is that α_2 AR activation can reduce nociceptive input into the spinal cord by direct inhibition of sensory neurons.

An extensive literature from biochemical, behavioral and electrophysiological studies exists supporting this principle. Biochemical studies in spinal cord slices, dorsal root ganglion (DRG) cell cultures and isolated spinal cord synaptosomes have demonstrated α_2 AR agonist-mediated inhibition of stimulus-evoked SP, calcitonin gene-related peptide (CGRP) or glutamate release (Bourgoin et al., 1993; Holz et al., 1988; Kamisaki et al., 1993; Li & Eisenach, 2001; Ono et al., 1991). Behavioral studies have shown that neurotoxic lesion of the nerve growth factor (NGF)-dependent population of primary afferent neurons (i.e. small diameter nociceptive neurons) results in the loss of clonidine-induced anti-allodynic activity (Paqueron et al., 2001). Electrophysiological studies in anesthetized rat preparations have shown that the α_2 AR agonists clonidine and dexmedetomidine inhibit afferent fiber-evoked responses of dorsal horn neurons in a dose-dependent manner (Sullivan et al., 1987; Sullivan et al., 1992b). Similarly, in spinal cord slice preparations, NE, clonidine and oxymetazoline all inhibit excitatory glutamatergic input from primary afferent neurons into the spinal cord (Kawasaki

et al., 2003; Pan et al., 2002). Finally, Sonohata et al. (2004) revealed using *in vivo* patch-clamp recording that NE inhibits the barrage of excitatory post-synaptic potentials in rat spinal cord neurons initiated by nociceptive stimuli applied to the hindlimb. Thus, the activation of α_2 ARs on and subsequent inhibition of primary afferent terminals is highly likely to play an important role in α_2 AR-mediated antinociception. Efforts to identify the role of each of the α_2 AR subtypes in this action are explained below.

α_{2A} AR: Several studies have detected mRNA encoding the α_{2A} AR in DRG neurons (Nicholas et al., 1993; Cho et al., 1997; Shi et al., 2000). Shi et al. reported that approximately 20% of DRG neurons expressed α_{2A} AR. These cells were mostly medium in size and about half of them co-expressed mRNA for CGRP (Shi et al., 2000). IHC studies similarly suggest that α_{2A} AR is expressed by DRG, with 54% of all DRG neurons expressing α_{2A} AR-ir (Gold et al., 1997). In a study evaluating the origin of α_{2A} AR-ir in spinal cord, α_{2A} AR-ir was significantly attenuated by both dorsal rhizotomy and neonatal capsaicin-treatment, suggesting that the majority of α_{2A} ARs in the spinal cord are on primary afferent terminals (Stone et al., 1998). Furthermore, extensive co-expression was observed between α_{2A} AR-ir and SP-ir and approximately 50% of α_{2A} AR-ir spinal elements also expressed CGRP, supporting not only the aforementioned study by Gold and colleagues but also the primary afferent origin of these fibers (Stone et al., 1998). The presence of functional α_2 ARs on the capsaicin-sensitive population of primary afferent fibers is supported by Li and Eisenach (2001) in which capsaicin-induced glutamate release was inhibited by the α_2 AR agonists NE, clonidine, dexmedetomidine and ST91 with a pharmacological profile suggestive of the α_{2A} AR subtype (Li & Eisenach, 2001). Very recently, we have completed an extensive study employing both structural and functional assessment of α_{2A} AR co-localization with delta opioid receptors and the neuropeptides SP and CGRP (Riedl et al. 2009). IHC analysis of rat spinal cord tissue and synaptosomes showed almost complete co-localization of α_{2A} AR, delta opioid receptors and SP down to the level of primary afferent terminals. Furthermore, functional analysis of depolarization-evoked release of CGRP from synaptosomes indicated that either α_{2A} AR or delta opioid receptor agonists could inhibit release and the combination was ~30-fold more potent, suggesting significant synergy at the level of synaptic terminals. The coincidence of structural and functional data makes a strong case for α_{2A} AR-delta opioid receptor synergy taking place in antinociception-relevant subcellular compartments in spinal cord.

α_{2B} AR: Studies evaluating the presence of α_{2B} AR mRNA in DRG neurons have yielded inconsistent results. Whereas one study detected mRNA on the majority of both large and small neurons (Gold et al., 1997), others report only very low levels in a few cells (Cho et al., 1997; Shi et al., 2000). The contribution of the α_{2B} AR to adrenergic modulation of primary afferent signaling is therefore unclear.

α_{2C} AR: Many DRG cell bodies are intensely labeled for α_{2C} AR mRNA (Leiphart et al., 2004; Nicholas et al., 1993; Nicholson et al., 2005; Shi et al., 2000) and greater than 50% of these co-express mRNA for CGRP (Shi et al., 2000). Similarly, an IHC study reported α_{2C} AR-ir in 54% of L5 DRG neurons, approximately half of which were also immunopositive for the capsaicin receptor TRPV1 (Ma et al., 2005). Thus, both *in situ* and IHC22 data suggest that at least half of all primary afferents express α_{2C} AR and of those, approximately half have the peptidergic/TRPV1-ir phenotype. Other studies suggest, however, that only a small proportion of the α_{2C} AR-ir in the spinal cord is of primary afferent origin (Birder & Perl, 1999; Stone et al., 1998). For example, α_{2C} AR-ir in the spinal cord does not co-localize with CGRP or isolectin B4 (IB4) binding, both markers of nociceptive primary afferent fibers, is not reduced by neonatal capsaicin treatment, and is only slightly diminished by dorsal rhizotomy (Olave & Maxwell, 2002; Stone et al., 1998). A possible explanation that reconciles these observations

is that while many DRG neurons express α_2C AR, the receptor protein may be preferentially retained by the soma and not trafficked extensively to the central terminals.

2.4 Modulation of spinal & DRG expression of α_2 AR after peripheral nerve injury

In contrast to opioids, α_2 AR agonists increase in potency and efficacy after peripheral nerve injury in animals (Luo et al., 1994; Poree et al., 1998; Xu et al., 1992) and humans (Eisenach et al., 1995). Changes in descending noradrenergic inhibition may contribute to chronic neuropathic pain. In normal animals, the presence of tonic inhibition can be demonstrated by treatment with an α_2 AR antagonist, which significantly increases the evoked responses of spinal neurons to low-intensity mechanical stimuli (Rahman et al., 2008). In the spinal nerve ligation model, however, this inhibition is lost (Rahman et al., 2008). As a result, responses to low threshold mechanical stimuli are increased, which may contribute to the tactile allodynia observed in this model. The loss of descending inhibition could have several mechanisms including decreased descending NE input or the loss of α_2 AR targets in the spinal cord. The former hypothesis is inconsistent with observations of increased TH-ir and D β H-ir fibers in the spinal cord ipsilateral to chronic constriction injury of the sciatic nerve (Ma & Eisenach, 2003). The regulation of each of the α_2 AR subtypes following peripheral nerve injury and the possible functional sequences of these changes are discussed below.

α_{2a} AR—The data regarding the regulation of primary afferent and spinal α_{2A} AR expression following peripheral nerve injury is mixed. In the DRG, total expression of α_{2A} AR mRNA is increased (Cho et al., 1997). However, whereas the total number of cells expressing α_{2A} AR mRNA or α_{2A} AR-ir is increased, the overall intensity of mRNA in each positive cell decreased by approximately 50% (Shi et al., 2000). In the spinal cord, one study reported no changes in α_{2a} AR mRNA (Shi et al., 1999) whereas another reported bilateral decreases in mRNA (Leiphart et al., 2003). The latter is consistent with observations that α_{2A} AR-ir is significantly decreased following sciatic nerve transection, chronic constriction injury and spinal nerve ligation (Stone et al., 1999). Pharmacological studies have shown that the analgesic target of intrathecal clonidine switches from α_{2a} AR to the α_{2b} AR or α_{2C} AR as its primary target following spinal nerve ligation (Duflo et al., 2002), suggesting that the α_{2a} AR may be down-regulated. In contrast, however, is a study indicating that α_2 AR activation, as measured by norepinephrine-stimulated [35 S]GTPgammaS binding, is increased following spinal nerve ligation with a pharmacological profile most consistent with the α_{2A} AR subtype. It is therefore not clear from currently available data if peripheral nerve injury results in a net loss or a net gain in α_{2a} AR or what the functional consequences of these changes may be.

α_{2B} AR—Expression of α_{2b} AR mRNA does not appear to be modulated by PNI in either superficial dorsal horn or DRG neurons (Cho et al., 1997; Shi et al., 1999). However, pharmacological evidence from one study (Leiphart et al., 2004) has suggested that the α_{2b} AR mediates spinal tizanidine efficacy following chronic constriction injury.

α_{2C} AR—The mRNA and immunohistochemical data regarding the impact of peripheral nerve injury on α_{2C} AR expression are not in agreement. mRNA studies indicate that α_{2C} AR mRNA is decreased in both DRG (Shi et al., 2000) (Cho et al., 1997) and spinal cord (Leiphart et al., 2003; Shi et al., 1999). In contrast, immunohistochemical studies report no change (Birder & Perl, 1999), complex upregulation of α_{2C} AR-ir in DRG (Ma et al., 2005) or increased expression in superficial dorsal horn (Stone et al., 1999). In the DRG, a reduction in the number of small, α_{2C} AR-ir neurons is accompanied by an increase in the number of medium and large, lightly stained cells, many of which co-expressed TRPV1 (Ma et al., 2005). This increase in α_{2C} AR-ir was associated with a corresponding 5-fold increase in the number of clonidine-inhibited DRG neurons that respond to capsaicin (Ma et al., 2005). Thus, increased expression of α_{2C} AR-ir on TRPV1-expressing neurons suggests that they may play a role in the analgesic

effects of clonidine following nerve injury. The switch from an α_{2A} AR- to an α_{2C} AR-mediated mechanism of action by clonidine following spinal nerve ligation (Dufflo et al., 2002) may therefore be explained by an increase in α_{2C} AR protein in the spinal cord, although not all the data support this hypothesis.

3.0.1 Introduction to Analgesic Synergy

The phenomenon of antinociceptive synergism resulting from co-application of opioid and α_2 AR agonists has been widely described. Synergistic drug interactions result in enhanced potency and/or efficacy when one agent is given together with another. Drug interactions are evaluated by a statistical method known as isobolographic analysis in which the actual potency of two drugs in combination is compared to that predicted in the absence of an interaction (Tallarida, 2001). Isobolographic analysis is considered the gold standard for the evaluation of drug interactions and only studies applying this standard are considered in this review. Co-application of synergistic partners offers the opportunity for reduced dose and side effect profiles compared to the drugs given alone. The potential for widening the analgesic therapeutic window drives the continued search for novel useful combinations of analgesic agents. For example, the therapeutic application of α_2 AR agonists either as monotherapeutics or as adjuvants (particularly with opioids and local anesthetics) continues to be the focus of recent clinical investigations (Sanders & Maze, 2007).

In addition to α_2 AR-opioid synergy, evidence for a synergistic interaction between α_2 AR subtypes exists. Selective pharmacological antagonism suggests that the observed synergistic interaction between α_2 AR agonists ST-91 and dexmedetomidine was due to actions at the α_{2C} AR and α_{2A} AR, respectively (Graham et al., 2000). Further, some recently described interactions between α_2 AR agonists have been revealed that were not predicted by prior pharmacological studies (Fairbanks et al., 2009). The use of α_2 AR mutant mice in this study combined with isobolographic analysis (Tallarida, 2001) revealed interactions likely between α_{2A} AR and α_{2C} AR that would otherwise evade detection. These specific combinations will be profiled in the individual sections below.

3.0.2 Introduction to Specific Ligands

Six α_2 AR agonists have received most attention over the past three decades: NE, clonidine, dexmedetomidine, brimonidine, ST-91 and moxonidine (Fig. 1). There is consensus that clonidine, brimonidine and dexmedetomidine exert their analgesic effects predominantly (though not exclusively) via the α_{2A} AR (Kable et al., 2000) whereas ST-91 (Stone et al., 2007b) and moxonidine (Fairbanks, 2002) evoke the participation of α_{2C} ARs. An extensive literature addresses interactions between these adrenergic agonists and opioid agonists in production of spinal antinociception; two more recent papers examine interactions between adrenergic agonists (Graham et al., 2000; Fairbanks et al., 2009). A majority of these papers use intrathecal administration to focus on the spinal site of action, and almost all find synergistic interactions between various adrenergic agonists and various opioid agonists (Drasner & Fields, 1988; Fairbanks et al., 2000; Fairbanks et al., 2002; Fairbanks & Wilcox, 1999b; Meert & De Kock, 1994; Monasky et al., 1990; Ossipov et al., 1990a; Ossipov et al., 2000; Ossipov et al., 1990c; Roerig et al., 1992; Stone et al., 1997b; Sullivan et al., 1987; Wilcox et al., 1987).

As mentioned in the General Introduction, a vast literature reports analgesia evoked by α_2 AR-selective and opioid receptor-selective agonists delivered as single agents; a subset of this literature describes synergistic interactions when the agents are applied as co-adjuvants. In contrast, few investigations have addressed the receptor subtypes mediating the actions of and interactions between these agonists when delivered either singly or as co-adjuvants. The advent of genetically altered mouse lines in the 1990s enabled evaluation of the receptor subtypes

activated by the gold standard α_2 AR-selective agonists when delivered singly or together with opioid receptor-selective agonists. The information acquired from such experiments highlights several important cautionary notations. First, delivery of a given agonist or agonist combination by different routes of administration can appear to show differential requirements for receptor subtypes in the pharmacological response. Second, reported *in vitro* binding affinities do not always correspond to potencies or efficacies determined by *in vivo* studies. Third, characterization of the pharmacological profile of a class of compounds requires broad-spectrum evaluation of multiple agonists from that class; extrapolation from the pharmacological profile of a single agonist may not be a reliable predictor of the profile of the class. Fourth, the pharmacological profile of two drugs delivered individually may not predict the pharmacology of the same two drugs delivered together. The remainder of this review will detail the pharmacological profile of the six aforementioned α_2 AR-agonists in normal and mutant mice when administered singly and in combination with other agonists.

3.1 Routes of Administration

The relationship between pharmacokinetics (drug distribution and clearance) and pharmacodynamics (drug effect) is sometimes overlooked in studies of drug action in the CNS. Failure to account for factors related to drug distribution could impact interpretation of results obtained through the use of a single route of administration. Both the efficacy and side effect profile of some α_2 AR agonists are substantively affected by the route of administration. For example, the role of the α_{2A} AR subtype in moxonidine-mediated sedation is dependent on route of administration. As shown in Figure 2, the α_2 AR agonist moxonidine was delivered intraperitoneally (Panel A), intrathecally (Panel B) and intracerebroventricularly (Panel C). The dependent measure in this case was sedation as measured by accelerating rotarod. The subjects evaluated were α_{2A} AR mutant mice and their wild type counterparts. As is evident, the potency of moxonidine did not differ between wildtype and α_{2A} AR mutant mice when delivered intraperitoneally. Were the results from the systemic study alone used to interpret the importance of the α_{2A} AR in moxonidine-evoked sedation, a premature conclusion would be drawn that the α_{2A} AR is not important to the effect. However, in stark contrast, when moxonidine is delivered centrally either intrathecally (Panel B) or intracerebroventricularly (Panel C), the sedative effect is not evident in α_{2A} AR mutant mice. These data clearly suggest a role for α_{2A} AR in centrally mediated sedation. This figure illustrates the importance of comprehensive experimental design that accounts for route of administration and the limited power of applying systemic routes of administration to evaluate effects mediated by actions in the CNS.

3.2 Mutant mouse lines

The multiplicity of NE's inhibitory receptor targets in spinal cord together with clonidine's demonstrated analgesic efficacy mandated a search for the relevant receptor subtypes involved. Unlike opioid receptors, which have a plethora of subtype-selective agonists, ligand selectivity across the three α_2 AR subtypes, α_{2A} AR, α_{2B} AR and α_{2C} AR, is limited (Jasper et al., 1998). The lack of highly subtype-selective agonists and antagonists has retarded progress in ascribing functional roles to the three receptor subtypes. Use of antagonists with ~100 fold selectivity for α_{2A} ARs (BRL44408) and α_{2B}/α_{2C} ARs (ARC239) has allowed some separation of the participation of the subtypes (Duflo et al., 2003; Duflo et al., 2002), but the most powerful identification of functional roles has resulted within the past decade from the use of genetically altered mouse lines: substitution of an asparagine residue with aspartate (D79N) resulting in functional knock-out of α_{2A} AR (Lakhani et al., 1997; Stone et al., 1998); knockout of α_{2B} AR and α_{2C} ARs (Link et al., 1996; Hunter et al., 1997; Kable et al., 2000). These studies of genetically altered mouse lines resulted in several major conclusions: spinal α_{2A} ARs mediate the antinociceptive effects of the majority of α_2 AR agonists (Stone et al., 1997b) while

supraspinal α_{2A} ARs mediate their sedative (Hunter et al., 1997) and hypotensive (Hunter et al., 1997; MacMillan et al., 1996) effects. In addition, some α_2 AR agonists recruit α_{2C} ARs to produce analgesic but not sedative effects, suggesting that the α_{2C} AR represents a viable analgesic drug target with reduced sedative liability.

3.3 Norepinephrine

That a unique relationship exists between the catecholamine-containing neurons of the CNS and the opioidergic system has been long appreciated (Cicero, 1974) and extensively investigated. The inhibitory effect of NE on the activation of dorsal horn neurons by noxious stimuli has been widely documented for decades (Engberg & Ryall, 1966; Headley et al., 1978; Belcher, 1978). Fibers descending from the brainstem, predominantly the locus coeruleus in the rostral pons, release NE in dorsal horn during episodes of stress or fear. This endogenous NE acts at spinal α_2 ARs, which couple predominantly through inhibitory G proteins, to inhibit transmission from nociceptive afferent fibers to secondary neurons. That part of this inhibitory action is mediated presynaptically on primary afferent terminals is supported by both anatomical and physiological data: dorsal rhizotomy or neonatal capsaicin ablates α_{2A} AR-like immunoreactivity (ir) on puncta in dorsal horn having the dimension of synaptic terminals (Stone et al., 1998); and NE inhibits release of the neuropeptides CGRP and SP (Takano et al., 1993) and nociceptive synaptic transmission from afferents to secondary neurons (Sonohata et al., 2004). In addition, however, NE also imposes postsynaptic inhibition on secondary dorsal horn neurons through hyperpolarization (Kawasaki et al., 2003), presumably via $G_{i/o}$ coupling to G protein-coupled inwardly rectifying potassium channels.

Like endogenous NE, exogenously administered NE, other natural non-selective agonists, and synthetic agonists selective for α_2 ARs also strongly and selectively (i.e. sensory vs. motor effects) inhibit nociceptive transmission via these same mechanisms. One of the earliest physiological studies of spinal cord adrenergic inhibition demonstrated that exogenous NE inhibited more than half of the spinal interneurons activated at short latency by cutaneous stimulation or at high latency (Engberg & Ryall, 1966); although this profile suggested sensory rather than motor neurons, there was no attempt to validate this distinction. Twelve years later, the selectivity of NE's inhibitory influence for nociceptive spinal cord neurons was demonstrated by two groups simultaneously (Belcher et al., 1978; Headley et al., 1978); interestingly, the sensory selectivity of NE exceeded that of the other major monoamine released by descending axons, serotonin. Using two thermal nociceptive assays in rats, Yaksh and colleagues (Reddy et al., 1980) compared the antinociceptive potencies of several intrathecally administered adrenergic agonists; the rank order of potencies was NE = epinephrine > α -methyl-NE > clonidine > phenylephrine = oxymetazoline; of these, only the synthetic agonist clonidine is selective for α_2 ARs. The following two decades saw several extensions to these early studies of noradrenergic action on nociceptive transmission: Hylden and Wilcox (1983) demonstrated that exogenous NE given intrathecally could inhibit SP-elicited nociceptive behaviors in mice in a manner that shows a significant participation by significant participation by α_{2A} AR (Fairbanks et al., 1999; Table 1). Additionally, NE potentiates morphine's inhibition almost ten-fold (Hylden & Wilcox, 1983); Roerig and colleagues (Roerig et al., 1992) extended this finding with isobolographic analysis and parallel studies of cocaine and clonidine (Table 2). Interestingly, the latter study found that NE, cocaine and clonidine synergized the most with the selective DOR agonist DPDPE followed by the mixed MOR/DOR agonist morphine. The selective MOR agonist DAMGO was subadditive with all three agonists; curiously, either the opioid antagonist naloxone or the α_2 AR antagonist idazoxan altered the interactions with DAMGO to be synergistic.

Endogenous NE participates in the analgesic efficacy of opioids. For example, administration of morphine directly into midbrain nuclei (i.e. PAG) results in spinal antinociception due to

release of both serotonin and NE in the spinal cord (Yaksh, 1979). Similarly, systemic administration of morphine increases NE levels in spinal superfusate (Taiwo et al., 1985), and intravenous administration of morphine increases concentrations of NE in both CSF and dorsal horn of sheep, providing further support for this model (Bouaziz et al., 1996). Concomitant delivery of opioids to the brain and spinal cord results in analgesic synergism (Yaksh & Rudy, 1978; Yeung & Rudy, 1980). That endogenous NE contributes to this synergy was inferred from the presence of NE in *in vivo* perfusate from spinal cord in the presence of monoamine uptake inhibitors and the reduction by the α_2 AR antagonist yohimbine of systemic morphine's analgesic potency (Taiwo et al., 1985). Subsequently, Wigdor and Wilcox (1987) demonstrated that the analgesic synergism produced by co-administration of morphine to the brain and spinal cord could be blocked by intrathecal injection of the $\alpha_{1/2}$ AR antagonist phentolamine, indicating the importance of midbrain-derived NE to this brain/spinal synergy. This observation was extended using isobolographic analysis and concurrent supraspinal/spinal administration of agonists selective for each of the three opioid receptor subtypes in mice (Roerig & Fujimoto, 1989); interestingly the mu opioid receptor agonist DAMGO resulted in a synergistic interaction whereas delta opioid receptor (DPDPE) and kappa opioid receptor agonists did not (see Table 2 for definitions). A more recent study found that concurrent administration of another delta opioid receptor agonist, deltorphin II, to the rostroventral medial medulla and intrathecal space yielded a synergistic interaction (Grabow et al., 1999); whether the different agonists used or the different supraspinal routes of administration accounts for the disparity with the results of Roerig and Fujimoto (1989) remains unclear. Collectively, these studies clearly document that systemically or supraspinally applied opioids evoke the concomitant release of NE at the spinal cord level and that this spinal NE contributes to the overall analgesic response. This opioid-induced NE release likely underlies the antinociceptive synergism produced by concomitant delivery of opioids supraspinally and intrathecally and represents an underappreciated ongoing mechanism contributing significantly to the analgesia evoked by systemically delivered opioids for chronic pain management.

The mechanism of action of tramadol, which is often used in management of chronic pain, is thought to exploit this spinal/supraspinal interaction. Tramadol combines very weak opioid agonist activity with monoamine (i.e. NE and 5HT) reuptake inhibition, which would potentiate monoamines released from descending terminals (Raffa et al., 1992). Consistent with this reasoning, systemic pre-treatment with either yohimbine (α_2 AR antagonist) or ritanserin (5HT-receptor antagonist) reduces tramadol-induced antinociception in rats (Raffa et al., 1992) and mice (Ide et al., 2006). These observations suggest that tramadol's opioid activity activates descending monoaminergic neurons and that its reuptake inhibitory activity potentiates the efficacy of the monoamines released in the spinal cord. Consistent with this conclusion is the observation that intrathecally applied heterocyclic antidepressants, particularly those with noradrenergic reuptake inhibition efficacy, produce acute antinociception after intrathecal administration (Hwang & Wilcox, 1987). More recently, mice deficient in the norepinephrine transporter showed enhanced morphine potency (Bohn et al., 2000), presumably through an increase in concentration and/or availability of NE in the spinal extracellular space, and mice lacking the NE synthesis enzyme D β H have reduced responsiveness to morphine (Jasmin et al., 2002). Collectively, these observations support the involvement of endogenous NE and α_2 ARs in opioid receptor-mediated analgesia (Raffa et al., 1992).

3.4 Clonidine

The selective α_2 AR agonist clonidine was introduced as an anti-hypertensive agent in the early 1970s with the rationale that inhibiting NE release from sympathetic terminals in the periphery would decrease sympathetic tone. That its effects were largely the result of actions in the brainstem established its utility as a sedative and analgesic in the mid to late 1970s (Paalzow, 1974). Paalzow (1978) subsequently showed that, like morphine, chronic clonidine produced

homologous tolerance. Gold and Kleber (1979) proposed that locus coeruleus (LC) hyperactivity contributed importantly to opiate dependence, and clonidine, which inhibits LC activity via α_2 AR autoreceptors on noradrenergic neurons, was adopted as a treatment for withdrawal from chronic opiates. In addition to that proposal, it is possible that replacement of opioid analgesic effects (presumably rendered tolerant) with α_2 AR-mediated analgesia also moderates the sensory component of withdrawal. The first studies of intrathecal delivery of clonidine were conducted by Yaksh and Reddy (1981), who observed elevation of shock titration thresholds in macaque. Shortly thereafter, epidurally delivered clonidine was reported to be analgesic in human (Tamsen & Gordh, 1984) and was evaluated clinically in neuropathic and cancer-associated (Eisenach et al., 1995; Eisenach et al., 1989) and post-operative pain (Mendez et al., 1990). Intrathecally delivered clonidine was also shown to be effective for treatment of pain in patients who have developed morphine tolerance in cases of intractable cancer pain (Coombs et al., 1985; van Essen et al., 1988). Several clinical studies also demonstrated the efficacy of intrathecal clonidine for treatment of post-operative (Filos et al., 1992) and neuropathic pain (Siddall et al., 2000). Clonidine was subsequently developed for epidural use in pain management. Currently the primary use as a pain management tool is as a spinal adjuvant for opioids (Ghafoor et al., 2007).

Although the binding affinity of clonidine does not differ appreciably among the three receptor subtypes (A, B, C) (Piletz et al., 1996), it is consistently observed that the analgesic efficacy of intrathecally (Fairbanks & Wilcox, 1999a) and systemically (Stone, unpublished observations) delivered clonidine is completely abolished in the D79N- α_{2a} AR functional knock-out mouse line in both the tail flick and substance P nociceptive tests (Table 1). The dependence of clonidine on α_{2a} AR activation for antinociceptive efficacy was a consistent observation across several assays and research groups (Altman et al., 1999; Hunter et al., 1997; Lakhani et al., 1997; MacMillan et al., 1996; Szot et al., 2004). Consistent with a critical role for the α_{2a} AR, clonidine's intrathecal potency in the tail flick test was either unchanged or very slightly reduced (2–5 fold) in α_{2C} AR-KO mice relative to their wildtype controls (Stone, unpublished observations, Table 1). On the other hand, in the SP nociceptive test, which shows higher sensitivity to opioid and α_2 AR inhibition, clonidine's intrathecal and systemic potencies were moderately reduced (2–5 fold) in α_{2C} knock-out mice (Fairbanks et al., 2009). Therefore, although clonidine appears to act primarily at α_{2a} ARs, some of its antinociceptive efficacy may be attributable to α_{2C} AR activation.

The synergistic relationship between opioid agonists and NE discussed in the previous section generalizes to clonidine. Spaulding and coworkers (1979) was the first to report that clonidine potentiated morphine-induced antinociception. Subsequent studies showed clonidine potentiation of morphine analgesia when given either systemically or intrathecally (Yaksh & Reddy, 1981). In human subjects, various pharmacodynamic parameters of an epidurally delivered morphine-clonidine combination were also assessed in a post-operative (pancreatic cancer) study (Rockemann et al., 1995). The study reported that a low dose combination (1/2 the dose of clonidine given alone combined with 1/25th the dose of morphine given alone) was equieffective with either drug given alone, enhanced analgesic onset dynamics (relative to the slower onset of morphine alone) and increased duration of action (relative to short-acting clonidine alone), demonstrating a positive interaction on a multiple measures. Similarly, a study by D'Angelo colleagues (1999) observed that epidural clonidine significantly prolonged the duration of action of an epidural sufentanil-bupivacaine combination; however, it is unknown whether this outcome resulted from a pharmacokinetic or a pharmacodynamic interaction.

Isobolographic analysis is widely recognized as an appropriate statistical test for validating multiplicative interactions (Black & Sang, 2005). Intrathecal combinations of clonidine with various opioids have been evaluated isobolographically in rats (Ossipov, 1990) and mice

(Fairbanks & Wilcox, 1999b; Roerig et al., 1992; Stone et al., 1997b) and consistently confirm a multiplicative (i.e. synergistic) interaction at the spinal level (Table 2). Interestingly, the morphine-clonidine combination's antinociceptive synergy after intrathecal administration is not accompanied by synergy in sedation as measured using the rotarod test (Stone, German, Fairbanks and Wilcox, submitted); for this one side effect, the therapeutic window of the individual agents increases from about unity to more than 40 when they are given together. Electrophysiologic studies have similarly demonstrated that intrathecal clonidine potentiated intrathecal morphine in the reduction of dorsal horn unit responses to afferent noxious stimulation (Omote et al., 1991; Wilcox et al., 1987). Eisenach and colleagues (Eisenach et al., 1994) applied the isobolographic approach to assess the interaction between fentanyl and clonidine in patients receiving post-operative analgesics for post-caesarean pain. The ED₅₀ value of the fentanyl-clonidine combination was 52% lower than the predicted theoretical additive ED₅₀ value; however, this apparent difference was not statistically significant, indicating an additive interaction. The authors suggested two potential reasons for this unexpected outcome: a species difference or excessive variability in the human data. (For a complete summary of the human studies of opioid-clonidine interactions please see review by Walker and colleagues (2002)). The utility of epidural clonidine when given alone as a therapeutic agent is equivocal in terms of duration of action, safety and efficacy. Therefore, general clinical experience suggests that spinally delivered clonidine is more useful as an adjuvant to opioids or opioid-local anesthetic combinations than epidural clonidine alone (Ghafoor et al., 2007). Clinical application of clonidine remains an area of interest, particularly when used as an adjuvant for pain management and particularly through the spinal routes of administration (Eisenach et al., 1998). As noted above, clonidine lacks efficacy when given intrathecally or systemically in mice lacking functional α_{2a} ARs. This lack of full efficacy precluded a full isobolographic analysis of clonidine-opioid interactions in this mouse strain. Nonetheless, single dose interaction assessment with opioids in D79N- α_{2a} ARs mice using a high systemic dose of clonidine (100 mg/kg) combined with morphine has not revealed any residual interaction. In contrast, isobolographic studies in α_{2C} AR-KO mice of the interaction between systemically administered clonidine and morphine indicate a role for the α_{2C} AR in clonidine-morphine analgesia as the synergistic interaction observed in wildtype mice is reduced to additivity in the α_{2C} AR-KO (Stone, Fairbanks, Kitto, Nguyen & Wilcox, unpublished observations).

3.5 Dexmedetomidine

Maze and colleagues introduced dexmedetomidine, the d-enantiomer of medetomidine, in 1988 (Segal et al., 1988) as an anesthetic-sparing α_2 AR agonist and subsequently characterized as not potentiating alfentanil's cardiorespiratory depression (Furst et al., 1990). This latter observation is important for both clinical and mechanistic reasons: clinically, use of the combination should reduce the doses of each needed for analgesia and thereby increase its therapeutic index; mechanistically, the agents may produce cardiorespiratory depression by distinct and non-interacting mechanisms, suggesting the possibility that combination with other opioid agonists would yield similar results.

The main clinical application of dexmedetomidine is for sedation and anesthesia (Belleville et al., 1992; Kamibayashi & Maze, 2000). However, like clonidine, intrathecally administered dexmedetomidine was shown to be antinociceptive in rat (Fisher et al., 1991; Kalso et al., 1991), sheep (Eisenach, et al., 1994), and mouse (Stone, et al., 1997b). Like clonidine, dexmedetomidine's binding affinity differs only moderately between the three receptor subtypes (A, B, C) (Piletz et al., 1996) with a minor preference (10-fold) for α_{2a} ARs (MacDonald et al., 1997). Also like clonidine, the analgesic efficacy of intrathecally delivered dexmedetomidine is substantially reduced (2000-fold) in the D79N- α_{2a} AR mutant line in the SP nociceptive test (Stone, et al., 1997b), underscoring its dependence on functional α_{2a} AR

receptors. Significant reduction in dexmedetomidine potency (100–400-fold) is also observed with systemic administration (Table 1) in D79N- α_{2A} AR relative to wildtype mice. Substantial reduction of dexmedetomidine's effect in D79N- α_{2A} AR (Hunter et al., 1997; Lakhani et al., 1997; Ma et al., 2004; Paris et al., 2003) or α_{2A} AR-KO (Altman et al., 1999; Lahdesmaki et al., 2004; Link et al., 1996) mice is a consistent observation across several research groups.

Consistent with the dependence on α_{2A} AR for full effect, the intrathecal potency of dexmedetomidine is reduced only 2-fold in α_{2C} AR-KO mice (Fairbanks et al., 2009). Similarly, dexmedetomidine continued to show neuroprotective efficacy in α_{2C} AR-KO mice (Paris et al., 2003) in contrast to the α_{2A} AR-D79N mice (Ma et al., 2004; Paris et al., 2003). Furthermore, dexmedetomidine potency following systemic administration is either unchanged in α_{2C} AR-KO mice (Malmberg et al., 2001) or moderately reduced (6-fold; Stone, Fairbanks, Kitto, Nguyen & Wilcox, unpublished observations); this pattern is very similar to that observed with clonidine. Interestingly, while dexmedetomidine shows no change in locomotor activity or monoamine turnover in α_{2C} AR-KO or α_{2C} AR-overexpressing (OE) mice, it showed decreased hypothermia in α_{2C} AR-KO and increased hypothermia in α_{2C} AR-OE mice (Sallinen et al., 1997). Therefore, while dexmedetomidine appears to primarily prefer the α_{2A} AR, evidence indicates some activity at α_{2C} ARs *in vivo*.

As with clonidine, the interactions between dexmedetomidine and various inhibitory G protein-coupled receptor agonists administered intrathecally have also been evaluated (Table 2). Sullivan and colleagues (Sullivan et al., 1992a) reported that combination of dexmedetomidine with morphine synergistically inhibited C-fiber-evoked responses in anesthetized rats. Meert and colleagues (1994) later showed that dexmedetomidine potentiated fentanyl-evoked antinociception when given systemically in rat. This result suggested an interaction of dexmedetomidine's receptor (presumably α_2 AR) with the mu opioid receptor. Such an interaction was later confirmed isobolographically in an experiment combining dexmedetomidine and endomorphin-2 (Joo et al., 2000). It was subsequently shown that dexmedetomidine synergizes with a delta opioid receptor agonist in rat (Grabow et al., 1999). Interestingly, one report evaluated the analgesic interaction between dexmedetomidine and a cannabinoid receptor agonist, CP55,940 (Tham et al., 2005); the combination produced a synergistic interaction in the hot plate but not the tail flick assay; the reason for this discrepancy is not fully understood. In addition to opioid and cannabinoid receptor agonists, dexmedetomidine has been evaluated for interactions with two other α_2 AR agonists, ST91 (Graham et al., 2000) and clonidine (Fairbanks et al., 2009). The first study rationalized that, because dexmedetomidine appeared to act primarily at α_{2A} ARs whereas ST91 appeared to act at non- α_{2A} AR subtypes, a synergistic interaction might be expected; in fact, a synergistic interaction was observed. The second study arose from an unexpected finding; clonidine and dexmedetomidine, co-administered intrathecally, demonstrated replicable and robust analgesic synergism even though both agonists appeared to largely require the α_{2A} AR for full potency and efficacy (Fairbanks et al., 2009). To further test the possibility of involvement of non- α_{2A} ARs in that interaction, cross-tolerance studies were performed; that the two drugs did not show cross-tolerance supported the involvement of distinct mechanisms of action. Finally, the dexmedetomidine-clonidine combination was evaluated in α_{2C} AR-KO mice and compared against the synergistic interaction observed in wildtype mice. The combination was synergistic in wildtype but not in the α_{2C} AR-KO mice (Fairbanks et al., 2009), suggesting a role for the α_{2C} AR in the synergistic interaction between clonidine and dexmedetomidine, despite the fact that both agonists appear to have strong preference for the α_{2A} AR subtype when given as single agents. As stated earlier, the clinical use of dexmedetomidine has largely been for sedation and anesthesia. However, the combination of intrathecal dexmedetomidine with bupivacaine has been reported as effective for pain control with efficacy comparable to that of the clonidine-bupivacaine combination (Kamibayashi & Maze, 2000). Furthermore, a recent case report describes the addition of intrathecally delivered dexmedetomidine together with morphine to

provide pain management in a morphine-tolerant cancer patient (Ugur et al., 2007). Therefore, both clonidine and dexmedetomidine result in significant analgesia when injected intrathecally both in animal models (Kalso et al., 1991; Stone et al., 1997; Takano & Yaksh, 1991) and humans (Kanazi et al., 2006; Ugur et al., 2007). The key difference between clonidine and dexmedetomidine in terms of development for analgesic therapy is in the prior toxicity studies. Prior to therapeutic application of any new drug or combination of drugs intended for spinal administration it is imperative that comprehensive completion of preclinical neurotoxicity studies (Yaksh and Collins, 1989) be conducted. Additionally, controlled Phase I clinical trials of both the drugs delivered as single agents (Yaksh and Allen, 2004) and the drugs delivered as the combination (Hood et al., 1995) is required (Chiari et al., 1998). Thorough conduct of toxicological evaluation of therapeutics developed for CNS delivery is essential (Eisenach et al., 1998; Eisenach and Yaksh, 2002). While the toxicology of intrathecally delivered clonidine has been widely established (Yaksh and Collins, 1989), the neurotoxicity of intrathecally delivered dexmedetomidine has not been comprehensively evaluated.

3.6 Brimonidine, (UK14,304, Bromoxidine)

Brimonidine was developed in the 1970s as a clonidine analog intended to lower blood pressure without evoking sedative side effects (Ashton & Rawlins, 1978). However, subsequent pre-clinical studies indicated a sedative action for brimonidine in rat (Buerkle & Yaksh, 1998; Hayes et al., 1986; Van Zwieten, 1988). The primary clinical indication for which brimonidine has been developed is the treatment of glaucoma due to its ability to lower intraocular pressure (Greenfield et al., 1997), presumably by interacting with α_{2a} AR and α_{2C} ARs on epithelial cells (Woldemussie et al., 2007). In contrast, its application as an analgesic has been primarily as a research tool, which will be the focus of this review's discussion of brimonidine. As is the case with other α_2 AR agonists, systemically administered brimonidine produces antinociception in both rat (Hayes et al., 1986) and mouse (Millan, 1992; Millan et al., 1994); intrathecally administered brimonidine is also antinociceptive in both rat (Uhlen et al., 1990) and mouse (Guo et al., 2003; Stone et al., 1997b; Wu et al., 1993). Like other α_2 AR agonists, brimonidine's binding affinity differs only very modestly among the three receptor subtypes (A, B, C) with a moderate preference (20-fold) for α_{2a} AR (MacDonald et al., 1997) versus α_{2C} AR. Unlike clonidine and dexmedetomidine, brimonidine's analgesic efficacy after intrathecal delivery is reduced only 250-fold in the D79N- α_{2a} AR mutant line, suggesting less dependence on α_{2a} AR than evidenced by the greater shifts observed with clonidine and dexmedetomidine (Table 1) (Fairbanks et al., 2000; Stone et al., 1997b). Substantial reduction of brimonidine's effect in D79N- α_{2a} AR (Lakhlani et al., 1996; Shafaroudi et al., 2005) or α_{2a} AR-KO (Shafaroudi et al., 2005) mice is a consistent observation across several research groups. For example, in the SP nociceptive assay, brimonidine potency was only reduced 250-fold in the D79N- α_{2a} AR compared to the over 1000-fold shift observed for dexmedetomidine and the complete loss of clonidine efficacy in the SP nociceptive test. Significant reduction in brimonidine potency (41-fold (SP test) or loss of efficacy (tail flick test)) is also observed with systemic administration in D79N- α_{2a} AR mice relative to wildtype (Table 1).

The pattern of brimonidine pharmacology in α_{2C} AR-KO mice is identical to that of clonidine and dexmedetomidine. The potency of intrathecal brimonidine is unaltered in α_{2C} AR-KO mice (tail flick test) (Stone, unpublished observations, Table 1). However, intrathecally or systemically administered brimonidine is moderately reduced in potency (2 fold) in α_{2C} AR-KO mice in the SP nociceptive assay. This pattern is very similar to that observed with clonidine. Therefore, while brimonidine appears to largely access α_{2a} ARs, evidence also exists for activity at α_{2C} ARs *in vivo*; this is also supported by recent pharmacological results (Chen et al., 2008) showing reversal of brimonidine's effects by an α_{2C} AR (but not an α_{2a} AR) receptor-selective antagonist (JP-1320).

Interactions between brimonidine and several inhibitory G protein-coupled receptor agonists administered intrathecally have also been evaluated in normal and α_2 AR mutant mouse lines (Table 2). Like other α_2 AR agonists, brimonidine produced antinociceptive synergy in normal mice when delivered intrathecally with either a mu (DAMGO) or delta (deltorphin II) opioid receptor-selective agonist. Both these interactions were reduced to additivity in D79N- α_{2a} AR mice, demonstrating an important role for the α_{2a} AR as a synergistic partner for both mu and delta opioid receptors (Stone et al., 1997b). An important aspect of these observations is that the remaining efficacy and potency of brimonidine in D79N- α_{2a} AR mice indicate a possible role for non- α_{2A} ARs in its action. A subsequent study (Guo et al., 2003) evaluated the combination of brimonidine with DPDPE, a delta opioid receptor-selective agonist, in mu opioid receptor-KO mouse lines (Guo et al., 2003). The brimonidine-DPDPE combination proved to be synergistic in both wildtype (C57BL/6) and mu opioid receptor-KO mice, providing further support for α_{2A} AR-delta opioid receptor as a synergistic receptor pair in the spinal cord. Finally, a broader study of α_2 AR agonist pairs failed to detect synergy between intrathecal brimonidine and NE, clonidine, dexmedetomidine and moxonidine (Fairbanks et al., 2009; Fairbanks, unpublished observations).

3.7 ST91

ST-91 (2-[2,6-diethylphenylamino]-2-imidazoline) was first described in the 1970s as a clonidine derivative with cardiovascular pharmacology similar to that of clonidine when delivered centrally, but a different pharmacological profile when delivered intravenously (Hoefke et al., 1975). Further, it has been noted that, unlike clonidine, ST-91 evokes minimal sedation. These differences were largely attributed to physicochemical and pharmacokinetic parameters (Hoefke et al., 1975; Scriabine et al., 1977). Yaksh and Reddy later showed for the first time that intrathecally delivered ST-91 evoked antinociceptive responses in macaque (Yaksh & Reddy, 1981) when delivered alone and in combination with morphine. As in the original cardiovascular studies, systemically administered ST-91 was inactive in the macaque assay of nociception (Yaksh & Reddy, 1981) as well as the hot plate nociceptive assay in mice (Millan & Colpaert, 1991). These data suggest that, unlike the other α_2 AR agonists, ST-91 has limited access to the CNS where both the sedative and analgesic effects are centered. Subsequent studies have extended an analgesic role for intrathecally delivered ST-91 in both rat (Danzebrink & Gebhart, 1990; Graham et al., 2000; Malmberg & Yaksh, 1993) and mouse (Stone et al., 2007). The interest for development of ST-91 for pain control has arisen in part from a substantial pharmacological literature using α_2 AR-selective receptor antagonists (prazosin, imiloxan and ARC 239) indicating that ST-91 appeared to rely on non- α_{2A} AR subtypes as the principal target to evoke antinociception (Duflo et al., 2002; Takano et al., 1992; Takano & Yaksh, 1992). This result is in stark contrast to the results described above for NE, clonidine, dexmedetomidine, and brimonidine. Like the other α_2 AR agonists, the binding affinity of ST91 does not differ appreciably among the three receptor subtypes (A, B, C) (Jasper et al., 1998). In contrast to the aforementioned α_2 AR receptor agonists, the analgesic efficacy of intrathecally delivered ST-91 is reduced only 3-fold in the D79N- α_{2a} AR mutant line in the SP nociceptive test; like the previously mentioned agonists, the potency of ST-91 was unaltered in α_{2C} AR-KO mice relative to their wildtype counterparts in this test (Stone et al., 2007). In all mouse lines, ST-91-induced antinociception was dose-dependently reversed by the selective α_2 AR receptor antagonist, SK&F86466, confirming the participation of α_2 ARs in the observed response. To our knowledge, the study by Stone and colleagues (2007) is the only evaluation of the compound's potency in genetically altered animals.

Interactions between ST-91 and several inhibitory G protein-coupled receptor agonists administered intrathecally have also been evaluated in rat (Graham et al., 2000; Malmberg & Yaksh, 1993; Monasky et al., 1990) and mouse (Stone et al., 2007, Table 2). Expanding on the observation that ST-91 potentiated the antinociceptive effect of morphine in the macaque shock

titration model (Yaksh & Reddy, 1981), Yaksh and colleagues confirmed that the morphine-ST91 combination interacts synergistically in the hot plate thermal nociceptive assay (Monasky et al., 1990) as well as the formalin assay (Malmberg & Yaksh, 1993) in rat when given intrathecally. To follow up on the proposal that ST-91 activates non- α_{2A} ARs, we evaluated the performance of the ST91-deltorphan II combination in D79N- α_{2A} AR and α_{2C} AR-KO mice and their respective wildtype counterparts. Surprisingly, the ST91-deltorphan II combination was synergistic in all lines of mice (Stone et al., 2007). That result indicates that neither the α_{2A} AR nor the α_{2C} AR is *required* to achieve ST91-deltorphan II analgesic synergism. There are two possible explanations: first, ST-91 may synergize with deltorphan II through activation of either α_{2A} AR or α_{2C} ARs; or second, ST-91 synergizes with deltorphan II through activation of the α_{2B} AR subtype, the anatomical distribution and spinal pharmacology of which remains minimally investigated.

3.8 Moxonidine

Moxonidine emerged in 1981 (Armah & Stenzel, 1981) as a clonidine derivative with a cardiovascular pharmacological profile distinct from that of clonidine, with an improved side effect profile (Plänitz, 1984) and with reduced rebound withdrawal (Ziegler et al., 1996). It was considered a potential advance over clonidine in that it reduced hypotensive effects in normotensive subjects (Macphee et al., 1992). It is approved for use in Europe for treatment of mild and moderate hypertension (Fenton et al., 2006). Oral once or twice daily immediate release moxonidine has been shown to be effective for control of hypertension in several clinical studies both as a monotherapy and as a combination adjuvant; post-marketing surveillance indicates that it is largely well tolerated (Fenton et al., 2006). One notable exception was a trial of sustained release moxonidine in congestive heart failure patients (MOXCON Trial (Cohn et al., 2003)). In that study, treatment with a sustained release moxonidine preparation was associated with excess short-term mortality and morbidity compared to placebo control. The reasons for that outcome are unclear.

Consistent with the profile of moxonidine as an α_2 AR receptor agonist, intrathecally delivered moxonidine evokes antinociceptive responses in mice when delivered alone (Fairbanks & Wilcox, 1999a) and in combination with morphine or deltorphan II (but not DAMGO) (Fairbanks & Wilcox, 1999b) in the tail flick test and in the SP nociceptive assays. Systemically administered moxonidine was also shown to inhibit Phase I and Phase II of the formalin response in rat with potency comparable to that of morphine (Shannon & Lutz, 2000). Like the aforementioned agonists, the binding affinity for moxonidine does not differ between the three receptor subtypes (A, B, C) (Piletz et al., 1996), lacking even a minor preference for one over the other two. In contrast to most other α_2 AR agonists, studies in genetically modified animals suggest that its antinociceptive effects *in vivo* are attributable to actions at more than one receptor subtype. Moxonidine dose-dependently evokes spinal antinociception in both D79N- α_{2A} AR mutant animals and α_{2C} AR KO animals; furthermore, these actions are blocked in both lines of mice by the α_2 AR-selective antagonist, SK&F86466 (Fairbanks & Wilcox, 1999a) (Fairbanks et al., 2002). These data suggest that moxonidine-evoked antinociception does not require either the α_{2A} AR (Fairbanks & Wilcox, 1999a) or α_{2C} AR (Fairbanks et al., 2002) subtype as the principal target to evoke antinociception but that its actions do require α_2 ARs. However, reductions in potency and/or efficacy in the absence of either α_{2A} ARs or α_{2C} ARs indicate that both receptors contribute to intrathecal moxonidine-induced antinociception.

Interestingly, the blood pressure-lowering effect of systemically administered moxonidine was, in fact, observed to be ablated in D79N- α_2A (Zhu et al., 1999) and α_2A KO (Tan et al., 2002) mice in a manner equivalent to that of clonidine (Table 1). Similarly, when delivered systemically, moxonidine does not show a change in analgesic potency in D79N- α_{2A} AR mice,

although it is largely reduced (SP test) or ablated (TF test) in α_2C AR KO mice (Stone, unpublished observations). Furthermore, the role of α_2A AR in moxonidine-induced sedation depends on the route of administration as moxonidine-induced sedation is lost in D79N- α_2A AR mice following systemic but not central delivery (Fig. 2, this review). Interestingly, when delivered systemically, moxonidine does not show a change in analgesic potency in D79N- α_2A AR mice, but is significantly attenuated in α_2C AR KO mice (Stone, unpublished observations). That profile is consistent with an important role of α_2C AR in moxonidine-evoked analgesia and highlights the important pharmacological differences observed with delivery of the same agent by different routes of administration.

As noted above, the effects of intrathecal combination of moxonidine with several opioids have been evaluated in mouse (Fairbanks et al., 2000; Fairbanks & Wilcox, 1999b). Specifically, we have observed that the moxonidine-morphine and moxonidine-deltorphin II combinations produce synergism in both the SP nociceptive assay (Fairbanks & Wilcox, 1999b) and spinal nerve ligation-evoked hyperalgesia (morphine-moxonidine, Fairbanks et al., 2000). We have also shown that analgesic synergism with opioids is retained in D79N- α_2A AR when given either intrathecally (Fairbanks & Wilcox, 1999b) or systemically (Stone, unpublished observations). In contrast, the interaction is reduced to additivity in α_2C AR KO mice (Fairbanks et al., 2002). These results illustrate two key points. First, the performance of a synergistic combination cannot be presumed to be aligned with the performance of each agonist given separately; although the reduction of moxonidine potency in D79N- α_2A AR mice is equivalent to that seen in α_2C AR-deficient mice, the interactions with other ligands were distinct in both mouse lines. Instead, the loss of opioid synergism was only observed in the α_2C AR KO mice, emphasizing the importance of testing each combination in all available KO lines. Second, the observation of lack of moxonidine-deltorphin II synergism in α_2C AR-deficient suggests a role of α_2C AR as a synergistic partner for delta opioid receptor, an aspect not completely appreciated by the prior literature. To our knowledge, these are the only studies of moxonidine-evoked antinociceptive synergy, particularly employing genetically altered mice.

4.5 Summary and Conclusions

Adrenergic agonists, including endogenous NE, produce antinociception in the spinal cord by action both on the presynaptic terminals of primary afferent neurons and by direct inhibition of spinal cord neurons. The former is more likely to be mediated by α_2A AR and the latter by α_2C AR, although the evidence for each is mixed. Whereas adrenergic inhibition is increased by peripheral nerve injury, the role of the individual subtypes in that phenomenon is not clear.

Similarly, despite some inconsistencies in the literature, pharmacological themes also emerge. Most α_2 AR agonists (NE, clonidine, dexmedetomidine, brimonidine) have a strong dependence on α_2A ARs, although binding to and complementary activities at α_2C ARs contribute to some antinociceptive activity. Two α_2 AR agonists, ST-91 and moxonidine, without manifesting a binding selectivity for α_2C AR, apparently produce antinociception by acting in an α_2A AR-independent manner, perhaps by acting at α_2C ARs. Dexmedetomidine seems to be unique in manifesting α_2A AR dependence in producing antinociception while showing an ability to cross over to non- α_2A ARs (possibly α_2C ARs) under some conditions. In terms of sedation and motor impairment, as opposed to antinociception, after intrathecal administration in mice, most of the aforementioned agonists have marginal therapeutic indices, roughly 1; the exception in our hands is moxonidine, whose therapeutic index with regard to this particular side effect in mice approaches 10 (Stone and Fairbanks, unpublished).

All or most of the α_2 AR agonists synergize, increasing potency ten- to one hundred-fold, with various opioid agonists in production of antinociception; this outcome is clearest and most often studied after intrathecal administration but also often observed after systemic

administration (Stone, unpublished). Preliminary evidence with the clonidine-morphine combination suggests that synergy is not manifest in a test of motor impairment (rotarod assay); this absence of synergy results in increased therapeutic index by more than ten-fold (Stone, German, Fairbanks & Wilcox, submitted).

Perhaps surprisingly, at least three α_2 AR agonist pairs interact synergistically in production of antinociception: dexmedetomidine-ST91 (Graham et al., 2000), clonidine-dexmedetomidine (Fairbanks et al., 2009) and clonidine-moxonidine (Stone, unpublished observations). These observations suggest a clinical role for α_2 AR agonist-agonist combination therapy (pending appropriate toxicity studies). Furthermore, these data support the hypothesis that α_2 AR agonists exert antinociceptive actions on multiple α_2 AR subtypes.

This review has focused largely on mechanisms of action of α_2 AR antinociception and interdrug synergy, without addressing the side effect profile of the agents or the combinations. Several papers address clonidine's utility as a monotherapy (Eisenach et al., 1996; Eisenach & Dewan, 1990; Eisenach et al., 1995; Eisenach et al., 1989a,b; Mendez et al., 1990) as well as a component of combination with spinal opioids (Eisenach et al., 1994; Siddall et al., 2000) and/or local anesthetics (Sites et al., 2003; Walker et al., 2002). This is not the case for most of the other adrenergic agonists, including dexmedetomidine, brimonidine, ST91 and moxonidine. Clinical application of these other agents or combinations awaits thorough toxicological studies in animals (Yaksh & Collins, 1989) followed by controlled clinical trials to establish safety and efficacy (Eisenach & Yaksh, 2002; Yaksh & Allen, 2004). Synergistic combinations require separate validation (Chiari & Eisenach, 1998; Hood et al., 1995). Preliminary evidence from our laboratories indicates that, with respect solely to the sedative/motor impairment side effect, clonidine, dexmedetomidine and ST91, given alone intrathecally, have therapeutic indices of about 1; moxonidine (Stone, unpublished) and the clonidine-morphine (Stone, German, Fairbanks & Wilcox, submitted) combination have therapeutic indices exceeding 10. Clinical exploitation of synergy between drugs acting at G protein-coupled receptor has the potential for retained efficacy at lower doses and reduced side effect profiles compared to the drugs given alone. Such a synergistic combination would likely manifest a greatly increased therapeutic index relative to that of either agent given singly.

Literature Cited

- Aantaa R, Marjamaki A, Scheinin M. Molecular pharmacology of alpha 2-adrenoceptor subtypes. *Ann Med* 1995;27(4):439–449. [PubMed: 8519505]
- Altman JD, Trendelenburg AU, MacMillan L, Bernstein D, Limbird L, Starke K, Kobilka BK, Hein L. Abnormal regulation of the sympathetic nervous system in alpha2A-adrenergic receptor knockout mice. *Mol Pharmacol* 1999;56(1):154–161. [PubMed: 10385696]
- Aoki C, Go CG, Venkatesan C, Kurose H. Perikaryal and synaptic localization of alpha 2A-adrenergic receptor-like immunoreactivity. *Brain Res* 1994;650(2):181–204. [PubMed: 7953684]
- Armah IB, Stenzel W. BE-5895, a new clonidine-type antihypertensive aminopyrimidine derivative. *Naunyn Schmiedeberg's Arch Pharmacol* 1981;316:R42.
- Ashton H, Rawlins MD. Central nervous system depressant actions of clonidine and UK-14,304: partial dissociation of EEG and behavioural effects. *Br J Clin Pharmacol* 1978;5(2):135–140. [PubMed: 619947]
- Belcher G, Ryall RW, Schaffner R. The differential effects of 5-hydroxytryptamine, noradrenaline and raphe stimulation on nociceptive and non-nociceptive dorsal horn interneurons in the cat. *Brain Res* 1978;151(2):307–321. [PubMed: 679011]
- Belleville JP, Ward DS, Bloor BC, Maze M. Effects of intravenous dexmedetomidine in humans. I. Sedation, ventilation, and metabolic rate. *Anesthesiology* 1992;77(6):1125–1133. [PubMed: 1361310]
- Birder LA, Perl ER. Expression of alpha2-adrenergic receptors in rat primary afferent neurones after peripheral nerve injury or inflammation. *J Physiol* 1999;515(Pt 2):533–542. [PubMed: 10050019]

- Black DR, Sang CN. Advances and limitations in the evaluation of analgesic combination therapy. *Neurology* 2005;65(12 Suppl 4):S3–S6. [PubMed: 16385102]
- Bodnar RJ. Supraspinal circuitry mediating opioid antinociception: antagonist and synergy studies in multiple sites. *J Biomed Sci* 2000;7(3):181–194. [PubMed: 10810236]
- Bohn LM, Xu F, Gainetdinov RR, Caron MG. Potentiated opioid analgesia in norepinephrine transporter knock-out mice. *J Neurosci* 2000;20(24):9040–9045. [PubMed: 11124980]
- Bouaziz H, Tong C, Yoon Y, Hood DD, Eisenach JC. Intravenous opioids stimulate norepinephrine and acetylcholine release in spinal cord dorsal horn. Systematic studies in sheep and an observation in a human. *Anesthesiology* 1996;84(1):143–154. [PubMed: 8572328]
- Bourgoin S, Pohl M, Mauborgne A, Benoliel JJ, Collin E, Hamon M, Cesselin F. Monoaminergic control of the release of calcitonin gene-related peptide- and substance P-like materials from rat spinal cord slices. *Neuropharmacology* 1993;32(7):633–640. [PubMed: 7689707]
- Budai D, Harasawa I, Fields HL. Midbrain periaqueductal gray (PAG) inhibits nociceptive inputs to sacral dorsal horn nociceptive neurons through alpha2-adrenergic receptors. *J Neurophysiol* 1998;80(5):2244–2254. [PubMed: 9819240]
- Buerkle H, Yaksh TL. Pharmacological evidence for different alpha 2-adrenergic receptor sites mediating analgesia and sedation in the rat. *Br J Anaesth* 1998;81(2):208–215. [PubMed: 9813525]
- Chen W, Song B, Marvizon JC. Inhibition of opioid release in the rat spinal cord by alpha2C adrenergic receptors. *Neuropharmacology* 2008;54(6):944–953. [PubMed: 18343461]
- Chiari A, Eisenach JC. Spinal anesthesia: mechanisms, agents, methods, and safety. *Reg Anesth Pain Med* 1998;23(4):357–362. [PubMed: 9690586]discussion 384–357
- Cho HJ, Kim DS, Lee NH, Kim JK, Lee KM, Han KS, Kang YN, Kim KJ. Changes in the alpha 2-adrenergic receptor subtypes gene expression in rat dorsal root ganglion in an experimental model of neuropathic pain. *Neuroreport* 1997;8(14):3119–3122. [PubMed: 9331925]
- Cicero TJ. Effects of alpha-adrenergic blocking agents on narcotic-induced analgesia. *Arch Int Pharmacodyn Ther* 1974;208(1):5–13. [PubMed: 4152529]
- Cohn JN, Pfeffer MA, Rouleau J, Sharpe N, Swedberg K, Straub M, Wiltse C, Wright TJ. Adverse mortality effect of central sympathetic inhibition with sustained-release moxonidine in patients with heart failure (MOXCON). *Eur J Heart Fail* 2003;5(5):659–667. [PubMed: 14607206]
- Coombs DW, Saunders RL, Lachance D, Savage S, Ragnarsson TS, Jensen LE. Intrathecal morphine tolerance: use of intrathecal clonidine, DADLE, and intraventricular morphine. *Anesthesiology* 1985;62(3):358–363. [PubMed: 3883851]
- D'Angelo R, Evans E, Dean LA, Gaver R, Eisenach JC. Spinal clonidine prolongs labor analgesia from spinal sufentanil and bupivacaine. *Anesth Analg* 1999;88(3):573–576. [PubMed: 10072008]
- Danzebrink RM, Gebhart GF. Antinociceptive effects of intrathecal adrenoceptor agonists in a rat model of visceral nociception. *J Pharmacol Expl Ther* 1990;253(2):698–705.
- Davis KD, Treede RD, Raja SN, Meyer RA, Campbell JN. Topical application of clonidine relieves hyperalgesia in patients with sympathetically maintained pain. *Pain* 1991;47(3):309–317. [PubMed: 1664508]
- Dogrul A, Uzbay IT. Topical clonidine antinociception. *Pain* 2004;111(3):385–391. [PubMed: 15363883]
- Drasner K, Fields HL. Synergy between the antinociceptive effects of intrathecal clonidine and systemic morphine in the rat. *Pain* 1988;32(3):309–312. [PubMed: 3362566]
- Duflo F, Conklin D, Li X, Eisenach JC. Spinal adrenergic and cholinergic receptor interactions activated by clonidine in postincisional pain. *Anesthesiology* 2003;98(5):1237–1242. [PubMed: 12717147]
- Duflo F, Li X, Bantel C, Pancaro C, Vincler M, Eisenach JC. Peripheral nerve injury alters the alpha2 adrenoceptor subtype activated by clonidine for analgesia. *Anesthesiology* 2002;97(3):636–641. [PubMed: 12218531]
- Eisenach JC, D'Angelo R, Taylor C, Hood DD. An isobolographic study of epidural clonidine and fentanyl after cesarean section. *Anesth Analg* 1994;79(2):285–290. [PubMed: 7639365]
- Eisenach JC, De Kock M, Klimscha W. alpha(2)-adrenergic agonists for regional anesthesia. A clinical review of clonidine (1984–1995). *Anesthesiology* 1996;85(3):655–674. [PubMed: 8853097]

- Eisenach JC, Dewan DM. Intrathecal clonidine in obstetrics: sheep studies. *Anesthesiology* 1990;72(4): 663–668. [PubMed: 1969716]
- Eisenach JC, DuPen S, Dubois M, Miguel R, Allin D. Epidural clonidine analgesia for intractable cancer pain. The Epidural Clonidine Study Group. *Pain* 1995;61(3):391–399. [PubMed: 7478682]
- Eisenach JC, Hood DD, Curry R. Intrathecal, but not intravenous, clonidine reduces experimental thermal or capsaicin-induced pain and hyperalgesia in normal volunteers. *Anesth Analg* 1998;87(3):591–596. [PubMed: 9728835]
- Eisenach JC, Lysak SZ, Viscomi CM. Epidural clonidine analgesia following surgery: phase I. *Anesthesiology* 1989a;71(5):640–646. [PubMed: 2817456]
- Eisenach JC, Rauck RL, Buzzanell C, Lysak SZ. Epidural clonidine analgesia for intractable cancer pain: phase I. *Anesthesiology* 1989b;71(5):647–652. [PubMed: 2817457]
- Eisenach JC, Shafer SL, Bucklin BA, Jackson C, Kallio A. Pharmacokinetics and pharmacodynamics of intraspinal dexmedetomidine in sheep. *Anesthesiology* 1994;80(6):1349–1359. [PubMed: 7912045]
- Eisenach JC, Yaksh TL. Safety in numbers: How do we study toxicity of spinal analgesics? *Anesthesiology* 2002;97(5):1047–1049. [PubMed: 12411784]
- Engberg I, Ryall RW. The inhibitory action of noradrenaline and other monoamines on spinal neurones. *J Physiol* 1966;185(2):298–322. [PubMed: 16992224]
- Fairbanks CA, Wilcox GL. Moxonidine, a selective α_2 -adrenergic and imidazoline receptor agonist, produces spinal antinociception in mice. *J Pharmacol Exp Ther* 1999a;290(1):403–412. [PubMed: 10381806]
- Fairbanks CA, Wilcox GL. Spinal antinociceptive synergism between morphine and clonidine persists in mice made acutely or chronically tolerant to morphine. *J Pharmacol Exp Ther* 1999b;288(3):1107–1116. [PubMed: 10027848]
- Fairbanks CA, Nguyen HO, Grocholski BM, Wilcox GL. Moxonidine, a selective imidazoline- α_2 -adrenergic receptor agonist, produces spinal synergistic antihyperalgesia with morphine in nerve-injured mice. *Anesthesiology* 2000;93(3):765–773. [PubMed: 10969310]
- Fairbanks CA, Posthumus IJ, Kitto KF, Stone LS, Wilcox GL. Moxonidine, a selective imidazoline/ α (2) adrenergic receptor agonist, synergizes with morphine and deltorphin II to inhibit substance P-induced behavior in mice. *Pain* 2000;84(1):13–20. [PubMed: 10601668]
- Fairbanks CA, Stone LS, Kitto KF, Nguyen HO, Posthumus IJ, Wilcox GL. α (2C)-Adrenergic receptors mediate spinal analgesia and adrenergic-opioid synergy. *J Pharmacol Exp Ther* 2002;300(1):282–290. [PubMed: 11752127]
- Fairbanks CA, Nguyen HO, Kitto KF, Stone LS, Wilcox GL. Clonidine and dexmedetomidine produce antinociceptive synergy in mouse spinal cord. *Anesthesiology* 2009;110(3):638–647. [PubMed: 19212267]
- Fenton C, Keating GM, Lyseng-Williamson KA. Moxonidine: a review of its use in essential hypertension. *Drugs* 2006;66(4):477–496. [PubMed: 16597164]
- Filos KS, Goudas LC, Patroni O, Polyzou V. Intrathecal clonidine as a sole analgesic for pain relief after cesarean section. *Anesthesiology* 1992;77(2):267–274. [PubMed: 1642346]
- Fisher B, Zornow MH, Yaksh TL, Peterson BM. Antinociceptive properties of intrathecal dexmedetomidine in rats. *Eur J Pharmacol* 1991;192(2):221–225. [PubMed: 1674472]
- Ghafoor VL, Epshteyn M, Carlson GH, Terhaar DM, Charry O, Phelps PK. Intrathecal drug therapy for long-term pain management. *Am J Health Syst Pharm* 2007;64(23):2447–2461. [PubMed: 18029950]
- Gold MS, Dastmalchi S, Levine JD. Alpha 2 adrenergic receptor subtypes in rat dorsal root and superior cervical ganglion neurons. *Pain* 1997;69(1–2):179–190. [PubMed: 9060029]
- Gold MS, Kleber HD. A rationale for opiate withdrawal symptomatology. *Drug Alcohol Depend* 1979;4(5):419–424. [PubMed: 228923]
- Grabow TS, Hurley RW, Banfor PN, Hammond DL. Supraspinal and spinal δ (2) opioid receptor-mediated antinociceptive synergy is mediated by spinal α (2) adrenoceptors. *Pain* 1999;83(1):47–55. [PubMed: 10506671]
- Graham BA, Hammond DL, Proudfit HK. Synergistic interactions between two α (2)-adrenoceptor agonists, dexmedetomidine and ST-91, in two substrains of Sprague-Dawley rats. *Pain* 2000;85(1–2):135–143. [PubMed: 10692612]

- Greenfield DS, Liebmann JM, Ritch R. Brimonidine: a new alpha2-adrenoreceptor agonist for glaucoma treatment. *J Glaucoma* 1997;6(4):250–258. [PubMed: 9264305]
- Guo TZ, Davies MF, Kingery WS, Patterson AJ, Limbird LE, Maze M. Nitrous oxide produces antinociceptive response via alpha2B and/or alpha2C adrenoceptor subtypes in mice. *Anesthesiology* 1999;90(2):470–476. [PubMed: 9952154]
- Guo TZ, Jiang JY, Buttermann AE, Maze M. Dexmedetomidine injection into the locus ceruleus produces antinociception. *Anesthesiology* 1996;84(4):873–881. [PubMed: 8638842]
- Guo XH, Fairbanks CA, Stone LS, Loh HH. DPDPE-UK14,304 synergy is retained in mu opioid receptor knockout mice. *Pain* 2003;104(1–2):209–217. [PubMed: 12855331]
- Guyenet PG, Stornetta RL, Riley T, Norton FR, Rosin DL, Lynch KR. Alpha 2A-adrenergic receptors are present in lower brainstem catecholaminergic and serotonergic neurons innervating spinal cord. *Brain Res* 1994;638(1–2):285–294. [PubMed: 7911062]
- Hayes AG, Skingle M, Tyers MB. Antagonism of alpha-adrenoceptor agonist-induced antinociception in the rat. *Neuropharmacology* 1986;25(4):397–402. [PubMed: 2872610]
- Headley PM, Duggan AW, Griersmith BT. Selective reduction by noradrenaline and 5-hydroxytryptamine of nociceptive responses of cat dorsal horn neurones. *Brain Res* 1978;145(1):185–189. [PubMed: 638777]
- Hoefke W, Kobinger W, Walland A. Relationship between activity and structure in derivatives of clonidine. *Arzneimittelforschung* 1975;25(5):786–793. [PubMed: 1242325]
- Holz, Gt; Dunlap, K.; Kream, RM. Characterization of the electrically evoked release of substance P from dorsal root ganglion neurons: methods and dihydropyridine sensitivity. *J Neurosci* 1988;8(2):463–471. [PubMed: 2448433]
- Hood DD, Eisenach JC, Tuttle R. Phase I safety assessment of intrathecal neostigmine methylsulfate in humans. *Anesthesiology* 1995;82(2):331–343. [PubMed: 7856891]
- Horvath G, Joo G, Dobos I, Klimscha W, Toth G, Benedek G. The synergistic antinociceptive interactions of endomorphin-1 with dexmedetomidine and/or S(+)-ketamine in rats. *Anesth Analg* 2001;93(4):1018–1024. [PubMed: 11574376]
- Hosohata Y, Vanderah TW, Burkey TH, Ossipov MH, Kovelowski, Kovelowski SoraI, Uhl GR, Zhang X, Rice KC, Roeske WR, Hruba VJ, Yamaura HI, Lai J, Porreca F. Delta-Opioid receptor agonists produce antinociception and [³⁵S]GTPγS binding in mu receptor knockout mice. *Eur J Pharmacol* 2000;388(3):241–248. [PubMed: 10675732]
- Howe JR, Yaksh TL, Tyce GM. Intrathecal 6-hydroxydopamine or cervical spinal hemisection reduces norepinephrine content, but not the density of alpha 2-adrenoceptors, in the cat lumbar spinal enlargement. *Neuroscience* 1987a;21(2):377–384. [PubMed: 3039400]
- Howe JR, Yaksh TL, Go VL. The effect of unilateral dorsal root ganglionectomies or ventral rhizotomies on alpha 2-adrenoceptor binding to, and the substance P, enkephalin, and neurotensin content of, the cat lumbar spinal cord. *Neuroscience* 1987b;21(2):385–394. [PubMed: 2441314]
- Huang Y, Stamer WD, Anthony TL, Kumar DV, St John PA, Regan JW. Expression of alpha(2)-adrenergic receptor subtypes in prenatal rat spinal cord. *Brain Res Dev Brain Res* 2002;133(2):93–104.
- Hunter JC, Fontana DJ, Hedley LR, Jasper JR, Lewis R, Link RE, Secchi R, Sutton J, Eglen RM. Assessment of the role of alpha2-adrenoceptor subtypes in the antinociceptive, sedative and hypothermic action of dexmedetomidine in transgenic mice. *Br J Pharmacol* 1997;122(7):1339–1344. [PubMed: 9421280]
- Hwang AS, Wilcox GL. Analgesic properties of intrathecally administered heterocyclic antidepressants. *Pain* 1987;28:343–355. [PubMed: 3494977]
- Hylden JL, Wilcox GL. Pharmacological characterization of substance P-induced nociception in mice: modulation by opioid and noradrenergic agonists at the spinal level. *J Pharmacol Exp Ther* 1983;226(2):398–404. [PubMed: 6192235]
- Ide S, Minami M, Ishihara K, Uhl GR, Sora I, Ikeda K. Mu opioid receptor-dependent and independent components in effects of tramadol. *Neuropharmacology* 2006;51(3):651–658. [PubMed: 16793069]
- Ishii H, Kohno T, Yamakura T, Ikoma M, Baba H. Action of dexmedetomidine on the substantia gelatinosa neurons of the rat spinal cord. *Eur J Neurosci* 2008;27(12):3182–3190. [PubMed: 18554299]

- Jasmin L, Boudah A, Ohara PT. Long-term effects of decreased noradrenergic central nervous system innervation on pain behavior and opioid antinociception. *J Comp Neurol* 2003;460(1):38–55. [PubMed: 12687695]
- Jasmin L, Tien D, Weinshenker D, Palmiter RD, Green PG, Janni G, Ohara PT. The NK1 receptor mediates both the hyperalgesia and the resistance to morphine in mice lacking noradrenaline. *Proc Natl Acad Sci U S A* 2002;99(2):1029–1034. [PubMed: 11805341]
- Jasper JR, Lesnick JD, Chang LK, Yamanishi SS, Chang TK, Hsu SA, Daunt DA, Bonhaus DW, Eglen RM. Ligand efficacy and potency at recombinant alpha2 adrenergic receptors: agonist-mediated [35S]GTPgammaS binding. *Biochem Pharmacol* 1998;55(7):1035–1043. [PubMed: 9605427]
- Joo G, Horvath G, Klimscha W, Kekesi G, Dobos I, Szikszay M, Benedek G. The effects of ketamine and its enantiomers on the morphine- or dexmedetomidine-induced antinociception after intrathecal administration in rats. *Anesthesiology* 2000;93(1):231–241. [PubMed: 10861167]
- Kable JW, Murrin LC, Bylund DB. In vivo gene modification elucidates subtype-specific functions of alpha(2)-adrenergic receptors. *J Pharmacol Exp Ther* 2000;293(1):1–7. [PubMed: 10734146]
- Kalso EA, Poyhia R, Rosenberg PH. Spinal antinociception by dexmedetomidine, a highly selective alpha 2-adrenergic agonist. *Pharmacol Toxicol* 1991;68(2):140–143. [PubMed: 1677190]
- Kamibayashi T, Maze M. Clinical uses of alpha2-adrenergic agonists. *Anesthesiology* 2000;93(5):1345–1349. [PubMed: 11046225]
- Kamisaki Y, Hamada T, Maeda K, Ishimura M, Itoh T. Presynaptic alpha 2 adrenoceptors inhibit glutamate release from rat spinal cord synaptosomes. *J Neurochem* 1993;60(2):522–526. [PubMed: 8093480]
- Kanazi GE, Aouad MT, Jabbour-Khoury SI, Al Jazzar MD, Alameddine MM, Al-Yaman RR, Bulbul M, Baraka AS. Effect of low-dose dexmedetomidine or clonidine on the characteristics of bupivacaine spinal block. *Acta Anaesthesiol Scand* 2006;50(2):222–227. [PubMed: 16430546]
- Karim F, Roerig SC. Differential effects of antisense oligodeoxynucleotides directed against g(zalpha) and g(oalpha) on antinociception produced by spinal opioid and alpha(2) adrenergic receptor agonists. *Pain* 2000;87(2):181–191. [PubMed: 10924811]
- Kawasaki Y, Kumamoto E, Furue H, Yoshimura M. Alpha 2 adrenoceptor-mediated presynaptic inhibition of primary afferent glutamatergic transmission in rat substantia gelatinosa neurons. *Anesthesiology* 2003;98(3):682–689. [PubMed: 12606912]
- Kingery WS, Guo TZ, Davies MF, Limbird L, Maze M. The alpha(2A) adrenoceptor and the sympathetic postganglionic neuron contribute to the development of neuropathic heat hyperalgesia in mice. *Pain* 2000;85(3):345–358. [PubMed: 10781908]
- Lahdesmaki J, Sallinen J, MacDonald E, Scheinin M. Alpha2A-adrenoceptors are important modulators of the effects of D-amphetamine on startle reactivity and brain monoamines. *Neuropsychopharmacology* 2004;29(7):1282–1293. [PubMed: 15039766]
- Lakhlani PP, Lovinger DM, Limbird LE. Genetic evidence for involvement of multiple effector systems in alpha 2A-adrenergic receptor inhibition of stimulus-secretion coupling. *Mol Pharmacol* 1996;50(1):96–103. [PubMed: 8700125]
- Lakhlani PP, Macmillan LB, Guo TZ, McCool BA, Lovinger DM, Maze M, Limbird LE. Substitution of a mutant alpha(2a)-adrenergic receptor via hit and run gene targeting reveals the role of this subtype in sedative, analgesic, and anesthetic-sparing responses in vivo. *Proc Nat Acad Sci U S A* 1997;94(18):9950–9955.
- Leiphart JW, Dills CV, Levy RM. Decreased spinal alpha2a- and alpha2c-adrenergic receptor subtype mRNA in a rat model of neuropathic pain. *Neurosci Lett* 2003;349(1):5–8. [PubMed: 12946573]
- Leiphart JW, Dills CV, Levy RM. Alpha2-adrenergic receptor subtype specificity of intrathecally administered tizanidine used for analgesia for neuropathic pain. *J Neurosurg* 2004;101(4):641–647. [PubMed: 15481719]
- Li X, Eisenach JC. alpha2A-adrenoceptor stimulation reduces capsaicin-induced glutamate release from spinal cord synaptosomes. *J Pharmacol Exp Ther* 2001;299(3):939–944. [PubMed: 11714880]
- Li X, Zhao Z, Pan HL, Eisenach JC, Paqueron X. Norepinephrine release from spinal synaptosomes: auto-alpha2-adrenergic receptor modulation. *Anesthesiology* 2000;93(1):164–172. [PubMed: 10861160]

- Link RE, Desai K, Hein L, Stevens ME, Chruscinski A, Bernstein D, Barsh GS, Kobilka BK. Cardiovascular regulation in mice lacking alpha2-adrenergic receptor subtypes b and c. *Science* 1996;273(5276):803–805. [PubMed: 8670422]
- Luo L, Puke MJ, Wiesenfeld HZ. The effects of intrathecal morphine and clonidine on the prevention and reversal of spinal cord hyperexcitability following sciatic nerve section in the rat. *Pain* 1994;58(2):245–252. [PubMed: 7816492]
- Ma D, Hossain M, Rajakumaraswamy N, Arshad M, Sanders RD, Franks NP, Maze M. Dexmedetomidine produces its neuroprotective effect via the alpha 2A-adrenoceptor subtype. *Eur J Pharmacol* 2004;502(1–2):87–97. [PubMed: 15464093]
- Ma W, Eisenach JC. Chronic constriction injury of sciatic nerve induces the up-regulation of descending inhibitory noradrenergic innervation to the lumbar dorsal horn of mice. *Brain Res* 2003;970(1–2):110–118. [PubMed: 12706252]
- Ma W, Zhang Y, Bantel C, Eisenach JC. Medium and large injured dorsal root ganglion cells increase TRPV-1, accompanied by increased alpha2C-adrenoceptor co-expression and functional inhibition by clonidine. *Pain* 2005;113(3):386–394. [PubMed: 15661448]
- MacDonald E, Kobilka BK, Scheinin M. Gene targeting-homing in on alpha-2 adrenoceptor-subtype function. *Trends Pharmacol Sci* 1997;18:211–219. [PubMed: 9227000]
- MacMillan LB, Hein L, Smith MS, Piascik MT, Limbird LE. Central hypotensive effects of the alpha2a-adrenergic receptor subtype. *Science* 1996;273(5276):801–803. [PubMed: 8670421]
- Macphee GJ, Howie CA, Elliott HL, Reid JL. A comparison of the haemodynamic and behavioural effects of moxonidine and clonidine in normotensive subjects. *Br J Clin Pharmacol* 1992;33(3):261–267. [PubMed: 1576046]
- Malmberg AB, Hedley LR, Jasper JR, Hunter JC, Basbaum AI. Contribution of $\alpha 2$ receptor subtypes to nerve injury-induced pain and its regulation by dexmedetomidine. *Br J Pharmacol* 2001;132:1827–1836. [PubMed: 11309255]
- Malmberg AB, Yaksh TL. Pharmacology of the spinal action of ketotrolac, morphine, ST-91, U-50488 and L-PIA on the formalin test and an isobolographic analysis of the NSAID interaction. *Anesthesiology* 1993;79:1276–1279.
- Meert TF, De Kock M. Potentiation of the analgesic properties of fentanyl-like opioids with alpha 2 adrenoceptor agonists in rats. *Anesthesiology* 1994;81(3):677–688. [PubMed: 7916547]
- Mendez R, Eisenach JC, Kashtan K. Epidural clonidine analgesia after cesarean section. *Anesthesiology* 1990;73(5):848–852. [PubMed: 2240674]
- Millan MJ. Evidence that an alpha 2A-adrenoceptor subtype mediates antinociception in mice. *Eur J Pharmacol* 1992;215(2–3):355–356. [PubMed: 1356794]
- Millan MJ, Colpaert FC. Alpha 2 receptors mediate the antinociceptive action of 8-OH-DPAT in the hot-plate test in mice. *Brain Res* 1991;539(2):342–346. [PubMed: 1675912]
- Millan MJ, Bervoets K, Rivet JM, Widdowson P, Renouard A, Le Marouille-Girardon S, Gorbett A. Multiple alpha-2 adrenergic receptor subtypes. II. Evidence for a role of rat R alpha-2A adrenergic receptors in the control of nociception, motor behavior and hippocampal synthesis of noradrenaline. *J Pharmacol Exp Ther* 1994;270(3):958–972. [PubMed: 7932208]
- Monasky MS, Zinsmeister AR, Stevens CW, Yaksh TL. Interaction of intrathecal morphine and ST-91 on antinociception in the rat: dose-response analysis, antagonism and clearance. *J Pharmacol Exp Ther* 1990;254(2):383–392. [PubMed: 1974633]
- Nazarian A, Christianson CA, Hua XY, Yaksh TL. Dexmedetomidine and ST-91 analgesia in the formalin model is mediated by alpha(2A)-adrenoceptors: a mechanism of action distinct from morphine. *Br J Pharmacol* 2008;155(7):1117–1126. [PubMed: 18846040]
- Nicholas AP, Pieribone V, Hokfelt T. Distributions of mRNAs for alpha-2 adrenergic receptor subtypes in rat brain: an in situ hybridization study. *J Comp Neurol* 1993;328(4):575–594. [PubMed: 8381444]
- Nicholas AP, Hokfelt T, Pieribone VA. The distribution and significance of CNS adrenoceptors examined with in situ hybridization. *Trends Pharmacol Sci* 1996;17(7):245–255. [PubMed: 8756183]
- Nicholson R, Dixon AK, Spanswick D, Lee K. Noradrenergic receptor mRNA expression in adult rat superficial dorsal horn and dorsal root ganglion neurons. *Neurosci Lett* 2005;380(3):316–321. [PubMed: 15862909]

- North RA, Yoshimura M. The actions of noradrenaline on neurones of the rat substantia gelatinosa in vitro. *J Physiol* 1984;349:43–55. [PubMed: 6145790]
- Olave MJ, Maxwell DJ. An investigation of neurones that possess the alpha 2C-adrenergic receptor in the rat dorsal horn. *Neuroscience* 2002;115(1):31–40. [PubMed: 12401319]
- Olave MJ, Maxwell DJ. Axon terminals possessing the alpha 2c-adrenergic receptor in the rat dorsal horn are predominantly excitatory. *Brain Res* 2003a;965(1–2):269–273. [PubMed: 12591145]
- Olave MJ, Maxwell DJ. Neurokinin-1 projection cells in the rat dorsal horn receive synaptic contacts from axons that possess alpha2C-adrenergic receptors. *J Neurosci* 2003b;23(17):6837–6846. [PubMed: 12890778]
- Omote K, Kitahata LM, Collins JG, Nakatani K, Nakagawa I. Interaction between opiate subtype and alpha-2 adrenergic agonists in suppression of noxiously evoked activity of WDR neurons in the spinal dorsal horn. *Anesthesiology* 1991;74(4):737–743. [PubMed: 1672579]
- Ono H, Mishima A, Ono S, Fukuda H, Vasko MR. Inhibitory effects of clonidine and tizanidine on release of substance P from slices of rat spinal cord and antagonism by alpha-adrenergic receptor antagonists. *Neuropharmacology* 1991;30(6):585–589. [PubMed: 1717870]
- Ossipov MH, Harris S, Lloyd P, Messineo E. An isobolographic analysis of the antinociceptive effect of systemically and intrathecally administered combinations of clonidine and opiates. *J Pharmacol Exp Ther* 1990a;255(3):1107–1116. [PubMed: 2262895]
- Ossipov MH, Harris S, Lloyd P, Messineo E, Lin BS, Bagley J. Antinociceptive interaction between opioids and medetomidine: systemic additivity and spinal synergy. *Anesthesiology* 1990b;73(6):1227–1235. [PubMed: 1978990]
- Ossipov MH, Lozito R, Messineo E, Green J, Harris S, Lloyd P. Spinal antinociceptive synergy between clonidine and morphine, U69593, and DPDPE: isobolographic analysis. *Life Sci* 1990c;47(16):PL71–PL76. [PubMed: 2250556]
- Ossipov MH, Hong Sun T, Malan P Jr, Lai J, Porreca F. Mediation of spinal nerve injury induced tactile allodynia by descending facilitatory pathways in the dorsolateral funiculus in rats. *Neurosci Lett* 2000;290(2):129–132. [PubMed: 10936694]
- Paalzow L. Analgesia produced by clonidine in mice and rats. *J Pharmac & Pharmacol* 1974;26(5):361–363.
- Paalzow G. Development of tolerance to the analgesic effect of clonidine in rats cross-tolerance to morphine. Naunyn-Schmiedeberg's. *Arch Pharmacol* 1978;3041–3044.
- Pan YZ, Li DP, Pan HL. Inhibition of Blutamatergic Synaptic Input to Spinal Lamina II_o Neurons by Presynaptic alpha-2-Adrenergic Receptors. *J. Neurophysiol* 2002;87:1938–1947. [PubMed: 11929913]
- Paqueron X, Li X, Eisenach JC. P75-expressing elements are necessary for anti-allodynic effects of spinal clonidine and neostigmine. *Neuroscience* 2001;102(3):681–686. [PubMed: 11226704]
- Paris A, Philipp M, Tonner PH, Steinfath M, Lohse M, Scholz J, Hein L. Activation of alpha 2B-adrenoceptors mediates the cardiovascular effects of etomidate. *Anesthesiology* 2003;99(4):889–895. [PubMed: 14508322]
- Philipp M, Brede M, Hein L. Physiological significance of alpha(2)-adrenergic receptor subtype diversity: one receptor is not enough. *Am J Physiol Regul Integr Comp Physiol* 2002;283(2):R287–R295. [PubMed: 12121839]
- Piletz JE, Zhu H, Chikkala DN. Comparison of ligand binding affinities at human I-1-Imidazoline binding sites and the high affinity state of alpha-2 adrenoceptor subtypes. *J Pharmacol Exp Ther* 1996;279(2):694–702. [PubMed: 8930173]
- Plänitz V. Crossover comparison of moxonidine and clonidine in mild to moderate hypertension. *Eur J Clin Pharmacol* 1984;27(2):147–152. [PubMed: 6499895]
- Poree LR, Guo TZ, Kingery WS, Maze M. The analgesic potency of dexmedetomidine is enhanced after nerve injury: a possible role for peripheral alpha2-adrenoceptors. *Anesth Analg* 1998;87(4):941–948. [PubMed: 9768799]
- Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. *J Pharmacol Exp Ther* 1992;260(1):275–285. [PubMed: 1309873]

- Rahman W, D'Mello R, Dickenson AH. Peripheral nerve injury-induced changes in spinal alpha(2)-adrenoceptor-mediated modulation of mechanically evoked dorsal horn neuronal responses. *J Pain* 2008;9(4):350–359. [PubMed: 18226963]
- Reddy SV, Yaksh TL. Spinal noradrenergic terminal system mediates antinociception. *Brain Res* 1980;189:391–401. [PubMed: 6245762]
- Reddy SV, Maderdrut JL, Yaksh TL. Spinal cord pharmacology of adrenergic agonist-mediated antinociception. *J Pharmacol Exp Ther* 1980;213:525–533. [PubMed: 6110767]
- Riedl MS, Schnell SA, Overland AC, Chabot-Doré AJ, Taylor AM, Ribeiro-da-Silva A, Elde RP, Wilcox GL, Stone LS. Coexpression of alpha 2A-adrenergic and delta-opioid receptors in substance P-containing terminals in rat dorsal horn. *J. Comp. Neurol* 2009;513:385–398. [PubMed: 19180644]
- Rockemann MG, Seeling W, Brinkmann A, Goertz AW, Hauber N, Junge J, Georgieff M. Analgesic and hemodynamic effects of epidural clonidine, clonidine/morphine, and morphine after pancreatic surgery--a double-blind study. *Anesth Analg* 1995;80(5):869–874. [PubMed: 7726426]
- Roerig SC. Decreased spinal morphine/clonidine antinociceptive synergism in morphine-tolerant mice. *Life Sci* 1995;56(5):PL115–PL122. [PubMed: 7837926]
- Roerig SC, Fujimoto JM. Multiplicative interaction between intrathecally and intracerebroventricularly administered mu opioid agonists but limited interactions between delta and kappa agonists for antinociception in mice. *J Pharmacol Exp Ther* 1989;249(3):762–768. [PubMed: 2567350]
- Roerig SC, Howse KM. Omega-agatoxin IVA blocks spinal morphine/clonidine antinociceptive synergism. *Eur J Pharmacol* 1996;314(3):293–300. [PubMed: 8957249]
- Roerig SC, Lei S, Kitto K, Hylden JK, Wilcox GL. Spinal interactions between opioid and noradrenergic agonists in mice: multiplicativity involves delta and alpha-2 receptors. *J Pharmacol Exp Ther* 1992;262(1):365–374. [PubMed: 1378095]
- Rosin D, Zeng D, Stornetta R, Norton F, Riley T, Okusa M, Guyenet PG, Lynch KR. Immunohistochemical localization of alpha-2-A adrenergic receptors in catecholaminergic and other brainstem neurons in the rat. *Neuroscience* 1993;56:139–155. [PubMed: 7901804]
- Rosin DL, Talley EM, Lee A, Stornetta RL, Gaylinn BD, Guyenet PG, Lynch KR. Distribution of alpha 2C-adrenergic receptor-like immunoreactivity in the rat central nervous system. *J Comp Neurol* 1996;372(1):135–165. [PubMed: 8841925]
- Roudet C, Mouchet P, Feuerstein C, Savasta M. Normal distribution of alpha 2-adrenoceptors in the rat spinal cord and its modification after noradrenergic denervation: a quantitative autoradiographic study. *J Neurosci Res* 1994;39(3):319–329. [PubMed: 7869424]
- Sallinen J, Link RE, Haapalinna A, Viitamaa T, Kulatunga M, Sjöholm B, Macdonald E, Pelto-Huikko M, Leino T, Barsh GS, Kobilka BK, Scheinin M. Genetic alteration of alpha 2C-adrenoceptor expression in mice: influence on locomotor, hypothermic, and neurochemical effects of dexmedetomidine, a subtype-nonspecific alpha 2-adrenoceptor agonist. *Mol Pharmacol* 1997;51(1):36–46. [PubMed: 9016344]
- Sanders RD, Maze M. Alpha2-adrenoceptor agonists. *Curr Opin Investig Drugs* 2007;8(1):25–33.
- Sawamura S, Kingery WS, Davies MF, Agashe GS, Clark JD, Kobilka BK, Hashimoto T, Maze M. Antinociceptive action of nitrous oxide is mediated by stimulation of noradrenergic neurons in the brainstem and activation of [alpha]2B adrenoceptors. *J Neurosci* 2000;20(24):9242–9251. [PubMed: 11125002]
- Scheinin M, Lomasney JW, Hayden HD, Schambra UB, Caron MG, Lefkowitz RJ, Freneau RT Jr. Distribution of alpha 2-adrenergic receptor subtype gene expression in rat brain. *Brain Research. Mol Brain Res* 1994;21(1–2):133–149. [PubMed: 8164514]
- Scriabine A, Sweet CS, Ludden CT, Stavorski JM, Wenger HC, Bohidar NR. Some cardiovascular effects of ST-91 and clonidine. *Eur J Pharmacol* 1977;43(4):333–341. [PubMed: 18355]
- Seybold VS, Elde RP. Receptor autoradiography in thoracic spinal cord: correlation of neurotransmitter binding sites with sympathoadrenal neurons. *J Neurosci* 1984;4(10):2533–2542. [PubMed: 6149274]
- Shafaroudi MM, McBride M, Deighan C, Wokoma A, Macmillan J, Daly CJ, McGrath JC. Two “knockout” mouse models demonstrate that aortic vasodilatation is mediated via alpha2a-adrenoceptors located on the endothelium. *J Pharmacol Exp Ther* 2005;314(2):804–810. [PubMed: 15878998]

- Shannon HE, Lutz EA. Effects of the I-1 imidazoline/alpha(2)-adrenergic receptor agonist moxonidine in comparison with clonidine in the formalin test in rats. *Pain* 2000;85(1-2):161-167. [PubMed: 10692615]
- Shi TJ, Winzer-Serhan U, Leslie F, Hokfelt T. Distribution of alpha2-adrenoceptor mRNAs in the rat lumbar spinal cord in normal and axotomized rats. *Neuroreport* 1999;10(13):2835-2839. [PubMed: 10511449]
- Shi TS, Winzer-Serhan U, Leslie F, Hokfelt T. Distribution and regulation of alpha(2)-adrenoceptors in rat dorsal root ganglia. *Pain* 2000;84(2-3):319-330. [PubMed: 10666537]
- Siddall PJ, Molloy AR, Walker S, Mather LE, Rutkowski SB, Cousins MJ. The efficacy of intrathecal morphine and clonidine in the treatment of pain after spinal cord injury. *Anesth Analg* 2000;91(6):1493-1498. [PubMed: 11094007]
- Sites BD, Beach M, Biggs R, Rohan C, Wiley C, Rassias A, Gregory J, Fanciullo G. Intrathecal clonidine added to a bupivacaine-morphine spinal anesthetic improves postoperative analgesia for total knee arthroplasty. *Anesth Analg* 2003;96(4):1083-1088. [PubMed: 12651665]table of contents
- Sonohata M, Furue H, Katafuchi T, Yasaka T, Doi A, Kumamoto E, Yoshimura M. Actions of noradrenaline on substantia gelatinosa neurones in the rat spinal cord revealed by in vivo patch recording. *J Physiol* 2004;555(Pt 2):515-526. [PubMed: 14673188]
- Sora I, Funada M, Uhl GR. The mu-opioid receptor is necessary for [D-Pen(2),D-Pen(5)]enkephalin-induced analgesia. *Eur J Pharmacol* 1997;324(2-3):R1-R2. [PubMed: 9145787]
- Spaulding TC, Fielding S, Venafro SS, Lal H. Antinociceptive activity of clonidine and its potentiation of morphine analgesia. *Eur J Pharmacol* 1979;58(1):19-25. [PubMed: 499334]
- Stone LS, Broberger C, Vulchanova L, Wilcox GL, Hokfelt T, Riedl MS, Elde R. Differential distribution of alpha2A and alpha2C adrenergic receptor immunoreactivity in the rat spinal cord. *J. Neurosci* 1998;18(15):5928-5937. [PubMed: 9671679]
- Stone LS, Fairbanks CA, Laughlin TM, Nguyen HO, Bushy TM, Wessendorf MW, Wilcox GL. Spinal analgesic actions of the new endogenous opioid peptides endomorphin-1 and -2. *Neuroreport* 1997a;8(14):3131-3135. [PubMed: 9331928]
- Stone LS, MacMillan LB, Kitto KF, Limbird LE, Wilcox GL. The alpha2a adrenergic receptor subtype mediates spinal analgesia evoked by alpha2 agonists and is necessary for spinal adrenergic-opioid synergy. *J Neurosci* 1997b;17(18):7157-7165. [PubMed: 9278550]
- Stone LS, Vulchanova L, Riedl MS, Wang J, Williams FG, Wilcox GL, Elde R. Effects of peripheral nerve injury on alpha-2A and alpha-2C adrenergic receptor immunoreactivity in the rat spinal cord. *Neuroscience* 1999;93(4):1399-1407. [PubMed: 10501465]
- Stone LS, Kitto KF, Eisenach JC, Fairbanks CF, Wilcox GL. ST91-Mediated Spinal antinociception and synergy with opioids persists in the absence of Functional alpha-2A- or alpha-2C- adrenergic receptors. *J Pharmacol Expt Ther* 2007;323(3):899-906.
- Sullivan AF, Dashwood MR, Dickenson AH. Alpha 2-adrenoceptor modulation of nociception in rat spinal cord: location, effects and interactions with morphine. *Eur J Pharmacol* 1987;138(2):169-177. [PubMed: 3040431]
- Sullivan AF, Kalso EA, McQuay HJ, Dickenson AH. Evidence for the involvement of the mu but not delta opioid receptor subtype in the synergistic interaction between opioid and alpha 2 adrenergic antinociception in the rat spinal cord. *Neurosci Lett* 1992a;139(1):65-68. [PubMed: 1357605]
- Sullivan AF, Kalso EA, McQuay HJ, Dickenson AH. The antinociceptive actions of dexmedetomidine on dorsal horn neuronal responses in the anaesthetized rat. *Eur J Pharmacol* 1992b;215(1):127-133. [PubMed: 1355441]
- Szot P, Lester M, Laughlin ML, Palmiter RD, Liles LC, Weinshenker D. The anticonvulsant and proconvulsant effects of alpha2-adrenoreceptor agonists are mediated by distinct populations of alpha2A-adrenoreceptors. *Neuroscience* 2004;126(3):795-803. [PubMed: 15183527]
- Taiwo YO, Fabian A, Pazoles CJ, Fields HL. Potentiation of morphine antinociception by monoamine reuptake inhibitors in the rat spinal cord. *Pain* 1985;21(4):329-337. [PubMed: 4000684]
- Takano Y, Yaksh TL. Relative efficacy of spinal alpha-2 agonists, dexmedetomidine, clonidine and ST-91, determined in vivo by using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, an irreversible antagonist. *J Pharmacol Exp Ther* 1991;258(2):438-446. [PubMed: 1678011]

- Takano Y, Yaksh TL. Characterization of the pharmacology of intrathecally administered alpha-2 agonists and antagonists in rats. *J Pharmacol Exp Ther* 1992;261(2):764–772. [PubMed: 1349648]
- Takano Y, Takano M, Yaksh TL. The effect of intrathecally administered imiloxan and WB4101: possible role of alpha 2-adrenoceptor subtypes in the spinal cord. *Eur J Pharmacol* 1992;219(3):465–468. [PubMed: 1358645]
- Takano M, Takano Y, Yaksh TL. Release of calcitonin gene-related peptide (CGRP), substance P (SP), and vasoactive intestinal polypeptide (VIP) from rat spinal cord: modulation by alpha 2 agonists. *Peptides* 1993;14(2):371–378. [PubMed: 7683402]
- Tallarida RJ. Drug synergism: Its detection and applications. *J Pharmacol Exp Ther* 2001;298(3):865–872. [PubMed: 11504778]
- Talley EM, Rosin DL, Lee A, Guyenet PG, Lynch KR. Distribution of alpha 2A-adrenergic receptor-like immunoreactivity in the rat central nervous system. *J Comp Neurol* 1996;372(1):111–134. [PubMed: 8841924]
- Tamsen A, Gordh T. Epidural clonidine produces analgesia [letter]. *Lancet* 1984;2(8396):231–232. [PubMed: 6146793]
- Tan CM, Wilson MH, MacMillan LB, Kobilka BK, Limbird LE. Heterozygous alpha(2A)-adrenergic receptor mice unveil unique therapeutic benefits of partial agonists. *Proc Nat Acad Sci U S A* 2002;99(19):12471–12476.
- Tham SM, Angus JA, Tudor EM, Wright CE. Synergistic and additive interactions of the cannabinoid agonist CP55,940 with mu opioid receptor and alpha2-adrenoceptor agonists in acute pain models in mice. *Br J Pharmacol* 2005;144(6):875–884. [PubMed: 15778704]
- Ugur F, Gulcu N, Boyaci A. Intrathecal infusion therapy with dexmedetomidine-supplemented morphine in cancer pain. *Acta Anaesthesiol Scand* 2007;51(3):388. [PubMed: 17155939]
- Uhlen S, Persson ML, Alari L, Post C, Axelsson KL, Wikberg JE. Antinociceptive actions of alpha 2-adrenoceptor agonists in the rat spinal cord: evidence for antinociceptive alpha 2-adrenoceptor subtypes and dissociation of antinociceptive alpha 2-adrenoceptors from cyclic AMP. *J Neurochem* 1990;55(6):1905–1914. [PubMed: 1977885]
- Unnerstall JR, Kopajtic TA, Kuhar MJ. Distribution of alpha 2 agonist binding sites in the rat and human central nervous system: analysis of some functional, anatomic correlates of the pharmacologic effects of clonidine and related adrenergic agents. *Brain Res* 1984;319(1):69–101. [PubMed: 6324960]
- van Essen EJ, Bovill JG, Ploeger EJ, Beerman H. Intrathecal morphine and clonidine for control of intractable cancer pain. A case report. *Acta Anaesthesiol Belg* 1988;39(2):109–112.
- Van Zwieten PA. Pharmacology of the alpha 2-adrenoceptor agonist rilmenidine. *Am J Cardiol* 1988;61(7):6D–14D.
- Walker SM, Goudas LC, Cousins MJ, Carr DB. Combination spinal analgesic chemotherapy: a systematic review. *Anesth Analg* 2002;95(3):674–715. [PubMed: 12198058]
- Wang R, Macmillan LB, Freneau RT Jr, Magnuson MA, Lindner J, Limbird LE. Expression of alpha 2-adrenergic receptor subtypes in the mouse brain: evaluation of spatial and temporal information imparted by 3 kb of 5' regulatory sequence for the alpha 2A AR-receptor gene in transgenic animals. *Neuroscience* 1996;74(1):199–218. [PubMed: 8843087]
- Wigdor S, Wilcox GL. Central and systemic morphine-induced antinociception in mice: Comparison of descending serotonergic and noradrenergic pathways. *J Pharmacol Exp Ther* 1987;242:290–295.
- Wikberg-Matsson A, Wikberg JE, Uhlen S. Identification of drugs subtype-selective for alpha 2A-, alpha 2B-, and alpha 2C-adrenoceptors in the pig cerebellum and kidney cortex. *Eur J Pharmacol* 1995;284(3):271–279. [PubMed: 8666009]
- Wilcox GL, Carlsson KH, Jochim A, Jurna I. Mutual potentiation of antinociceptive effects of morphine and clonidine on motor and sensory responses in rat spinal cord. *Brain Res* 1987;405(1):84–93. [PubMed: 3567599]
- Wild KD, Press JB, Raffa RB. Alpha2-adrenoceptors: can subtypes mediate selective analgesia? *Analgesia* 1994;1(1):15–25.
- Woldemussie E, Wijono M, Pow D. Localization of alpha 2 receptors in ocular tissues. *Vis Neurosci* 2007;24(5):745–756. [PubMed: 17986363]

- Wu H, McLoon S, Wilcox GL. Antinociception following implantation of mouse pituitary AtT-20 cells in rat spinal cord. *J. Neural Transpl Plast* 1993;4:15–26.
- Xu XJ, Puke MJ, Wiesenfeld HZ. The depressive effect of intrathecal clonidine on the spinal flexor reflex is enhanced after sciatic nerve section in rats. *Pain* 1992;51(2):145–151. [PubMed: 1362458]
- Yaksh TL. Direct evidence that spinal serotonin and noradrenaline terminals mediate the spinal antinociceptive effects of morphine in the periaqueductal gray. *Brain Res* 1979;160(1):180–185. [PubMed: 581478]
- Yaksh TL, Allen JW. The use of intrathecal midazolam in humans: a case study of process. *Anesth Analg* 2004;98(6):1536–1545. [PubMed: 15155302]
- Yaksh TL, Collins JG. Studies in animals should precede human use of spinally administered drugs. *Anesthesiology* 1989;70(1):4–6. [PubMed: 2912314]
- Yaksh TL, Reddy SV. Studies in the primate on the analgetic effects associated with intrathecal actions of opiates, alpha-adrenergic agonists and baclofen. *Anesthesiology* 1981;54(6):451–467. [PubMed: 6112935]
- Yaksh TL, Rudy TA. Narcotic analgesics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. *Pain* 1978;4(4):299–359. [PubMed: 25403]
- Yeung JC, Rudy TA. Multiplicative interaction between narcotic agonisms expressed at spinal and supraspinal sites of action as revealed by concurrent intrathecal and intracerebroventricular injections of morphine. *J. Pharmacol. Exp. Ther* 1980;215:633–642. [PubMed: 6893721]
- Zhu QM, Lesnick JD, Jasper JR, MacLennan SJ, Dillon MP, Eglen RM, Blue DR. Cardiovascular effects of rilmenidine, moxonidine and clonidine in conscious wild-type and D79N alpha2A-adrenoceptor transgenic mice. *Br J Pharmacol* 1999;126(6):1522–1530. [PubMed: 10217548]
- Ziegler D, Haxhiu MA, Kaan EC, Papp JG, Ernsberger P. Pharmacology of moxonidine, an I1-imidazoline receptor agonist. *J Cardiovasc Pharmacol* 1996;27:S26–S37. [PubMed: 8872297]

Norepinephrine

Clonidine

Dexmedetomidine

Brimonidine
(UK 14,304)

ST-91

Moxonidine

Figure 1. Structures of α_2 AR agonists discussed in this review

Figure 2. The effect of route of administration on moxonidine's dependence on the α_{2A} AR subtype in the rotarod test

Moxonidine-evoked impairment of rotarod performance in WT (closed squares) and α_{2A} -D79N (open squares) mice following intraperitoneal (A), intrathecal (B), or intracerebroventricular (C) delivery. Whereas the effects systemically delivered moxonidine are α_{2A} AR-independent, centrally administered moxonidine is completely α_{2A} AR dependent in the rotarod test of sedation and motor impairment.

Table 1

Decreased potency shift relative to wildtype controls

	$a_{2A}AR$			$a_{2C}AR$		
	Analgesia		Sedation	Analgesia		Sedation
	I.T.	I.P.	I.T.	I.P.	I.T.	I.P.
NE	800 ¹	-	-	-	-	-
Clonidine	<50% efficacy ^{1,2}	<50% efficacy ¹⁰	<50% efficacy ²	<50% efficacy ¹²	2 (SP) ²	<50% efficacy ²
Dexmedetomidine	<50% efficacy ² 2500 ³	<50% efficacy ^{6,7,8,9}	<50% efficacy ²	<50% efficacy ^{6,7}	2 ²	<50% efficacy ²
Brimonidine	250 ³	<50% efficacy (TF) ¹⁰ 40(SP) ¹⁰	-	-	-	-
ST91	3 ⁵	-	-	-	No Change ⁵	-
Moxonidine	2-4 ^{1,4}	No change ⁹	<50% efficacy ⁹	No change ^{7,9}	2 ⁴	No change ⁹

¹(Fairbanks & Wilcox, 1999), ²(Fairbanks et al., 2009), ³Stone et al., 1997b), ⁴(Fairbanks et al., 2002), ⁵(Hunter et al., 1997), ⁶(Lakhlani et al., 1997), ⁷(Malmberg et al., 2001), ⁸(Kingery et al., 2000), ⁹(Stone, unpublished observations)

Assays = TF = tail flick, SP = substance P

Routes: I.T. = intrathecal, I.P. = intraperitoneal.

Table 2
Summary of Spinal Alpha-2 Adrenergic Antinociceptive Interactions.

	Species/Route	Mor	Fent	DAMGO	DPDPE	Delt II	Dex	ST-91	Other
NE	Mouse I.T	Syn ¹	-	Sub ¹	Syn ¹	-	-	-	-
Clon	Mouse I.T	Syn ^{1,2,3,4,5,6,7,8}	-	Sub ¹ /Add ²	Syn ^{1,2}	Syn ⁹	Syn ¹⁰	-	Add (U50488H) ²
Clon	Rat I.T	Syn ^{11#} , ^{12,13} /Add ^{11*} , ^{11*} , ^{11*} (tail flick; *hot plate)	Syn ¹¹	-	Syn ¹²	-	-	-	Syn [#] /Add [*] (Meperidine) ¹¹ (tail flick; *hot plate) Syn (U69593) ¹²
Clon	Human (epidural)	-	Add ¹⁴	-	-	-	-	-	-
Dex	Rat I.T	Syn ¹⁵	Syn ¹⁶	-	-	Syn ¹⁷	-	Syn ¹⁸	Syn (Endo-2) ¹⁹ Syn (CPP55,940) ²⁰
Brim	Mouse I.T	-	-	Syn ²¹	Syn ²²	Syn ²¹	-	-	Add (Clon) ¹⁰
ST-91	Mouse I.T	-	-	-	-	Syn ²³	-	-	-
ST-91	Rat I.T	Syn ²⁴	-	-	-	-	Syn ²⁵	-	Syn (Ketoprofen) ²⁶
Mox	Mouse I.T	Syn ^{27,28}	-	Sub ²⁷	-	Syn ²⁷	-	-	-

¹(Roerig et al., 1992); ²(Roerig & Fujimoto, 1989); ³(Fairbanks & Wilcox, 1999); ⁴(Karim & Roerig, 2000); ⁵(Wei et al., 1996); ⁶(Wei & Roerig, 1998); ⁷(Roerig, 1995); ⁸(Roerig & Howse, 1996); ⁹Overland et al., unpublished observations; ¹⁰(Fairbanks et al., 2009); ¹¹(Ossipov et al., 1990); ¹²(Ossipov et al., 1990); ¹³(Ossipov et al., 1997); ¹⁴(Eisenach et al., 1994); ¹⁵(Sullivan et al., 1992); ¹⁶(Meert & De Kock, 1994); ¹⁷(Grabow et al., 1999); ¹⁸(Graham et al., 2000); ¹⁹(Horvath et al., 2001); ²⁰(Tham et al., 2005); ²¹(Stone et al., 2003); ²²(Guo et al., 2003); ²³(Stone et al., 2007); ²⁴(Monasky et al., 1990); ²⁵(Graham et al., 2000); ²⁶(Malmberg & Yaksh, 1993); ²⁷(Fairbanks et al., 2000); ²⁸(Fairbanks et al., 2000)

Abbreviations: I.T. = intrathecal; Clon, clonidine; Dex, dexmedetomidine; Brim, brimidine; Mox, moxonidine; Mor, morphine; Fent, fentanyl; DAMGO, D-Ala-Gly-(me)Phe-Gly-ol; DPDPE, D-Pen2-D-Pen5-enkephalin; Delt II, deltorphin II; U50488H and U69593, non-peptide kappa opioid agonists; Endo-2, endomorphin 2; CPP55,940, cannabinoid receptor agonist; Syn, synergistic; Add, additive;