British Journal of Pharmacology (2001) 132, 1827–1836

www.nature.com/bjp

Contribution of α_2 receptor subtypes to nerve injury-induced pain and its regulation by dexmedetomidine

*,[1,2]Annika B. Malmberg, [3]Linda R. Hedley, [3]Jeffrey R. Jasper, [3]John C. Hunter & [1,2]Allan I. Basbaum

[1]Department of Anatomy, W.M. Keck Center for Integrative Neuroscience, University of California, San Francisco, California, CA 94143, U.S.A.; [2]Department of Physiology, W.M. Keck Center for Integrative Neuroscience, University of California, San Francisco, California, CA 94143, U.S.A. and [3]Neurobiology Unit, Roche Bioscience, Palo Alto, California, CA 94304, U.S.A.

1 There is evidence that noradrenaline contributes to the development and maintenance of neuropathic pain produced by trauma to a peripheral nerve. It is, however, unclear which subtype(s) of α adrenergic receptors (AR) may be involved. In addition to pro-nociceptive actions of AR stimulation, α_2 AR agonists produce antinociceptive effects.

2 Here we studied the contribution of the α_2 AR subtypes, α_{2A}, α_{2B} and α_{2C} to the development of neuropathic pain. We also examined the antinociceptive effect produced by the α_2 AR agonist dexmedetomidine in nerve-injured mice.

3 The studies were performed in mice that carry either a point (α_{2A}) or a null (α_{2B} and α_{2C}) mutation in the gene encoding the α_2 AR. To induce a neuropathic pain condition, we partially ligated the sciatic nerve and measured changes in thermal and mechanical sensitivity.

4 Baseline mechanical and thermal withdrawal thresholds were similar in all mutant and wild-type mice; and, after peripheral nerve injury, all mice developed comparable hypersensitivity (allodynia) to thermal and mechanical stimulation.

5 Dexmedetomidine reversed the allodynia at a low dose (3 μg kg^{-1}, s.c.) and produced antinociceptive effects at higher doses (10–30 μg kg^{-1}) in all groups except in α_{2A} AR mutant mice. The effect of dexmedetomidine was reversed by intrathecal, but not systemic, injection of the α_2 AR antagonist RS 42206.

6 These results suggest that neither α_{2A}, α_{2B} nor α_{2C} AR is required for the development of neuropathic pain after peripheral nerve injury, however, the spinal α_{2A} AR is essential for the antinociceptive effects of dexmedetomidine.

British Journal of Pharmacology (2001) **132,** 1827–1836

Keywords: α adrenergic receptors; peripheral nerve injury; neuropathic pain; thermal and mechanical allodynia; antinociception; guanethidine sympathectomy

Abbreviations: AR, adrenergic receptors; DRG, dorsal root ganglia; i.p., intraperitoneal; s.c., subcutaneous; +/+, wild-type mice; −/−, mutant mice

Introduction

Injury to a peripheral nerve can lead to a pain syndrome that is characterized by spontaneous pain, pain in response to normally innocuous stimuli (allodynia) and exaggerated pain in response to noxious stimuli (hyperalgesia). Although the etiology of neuropathic pain is not completely understood, in some patients it can be relieved by sympatholytic treatment (see Bonica, 1990). Under normal conditions, sensory neurons do not respond to sympathetic stimulation or to adrenergic agonists (Jänig *et al*., 1996). After injury to a peripheral nerve, however, sensory neurons can be activated by sympathetic stimulation and respond to local or circulating catecholamines (Devor & Jänig, 1981; Scadding, 1981; Sato & Perl, 1991). There are multiple sites where the abnormal interaction between the sensory and sympathetic nervous systems occurs, including damaged axons at the nerve injury site (Devor & Jänig, 1981), uninjured cutaneous nociceptors that innervate partially denervated skin (Ali *et al*., 1999) and in the dorsal root ganglion, where sprouting of sympathetic efferents around large diameter cell bodies has been described (McLachlan *et al*., 1993; Devor *et al*., 1994; Michaelis *et al*., 1996).

With a view to developing new therapies to treat these conditions, studies in both patients and in animal models of neuropathic pain have addressed the contribution of adrenergic receptor blockers. Several studies reported that the non-selective α AR antagonist, phentolamine, can reduce nerve injury-associated allodynia and hyperalgesia (Arner, 1991; Raja *et al*., 1991; Kim *et al*., 1993; Tracey *et al*., 1995, but see Ringkamp *et al*., 1999a). Although primate models and clinical studies indicate that the α_1 AR is critical to the sympathetic-sensory coupling (Davis *et al*., 1991; Drummond *et al*., 1996; Ali *et al*., 1999), several rodent studies have implicated the α_2 AR (Tracey *et al*., 1995; Xie *et al*., 1995; Chen *et al*., 1996).

Three different α_2 AR subtypes, α_{2A}, α_{2B} and α_{2C} have been identified (see Bylund *et al*., 1994). Immunocytochemical and

*Author for correspondence at: NeurogesX, Inc., 969C Industrial Road, San Carlos, CA 94070-4117, U.S.A.
Email: amalmberg@neurogesx.com

in situ hybridization studies indicate that all subtypes are located in regions associated with the processing of nociceptive information, including the superficial layers of the spinal dorsal horn (Rosin *et al*., 1993; 1996; Stone *et al*., 1998) and the dorsal root ganglion (Nicholas *et al*., 1993; Gold *et al*., 1997). The purpose of the present study was to examine the contribution of the different α_2 AR subtypes to the development of neuropathic pain. Because selective antagonists to these receptor subtypes are not readily available, we examined mice that carry either a point (α_{2A} AR; MacMillan *et al*., 1996) or null (α_{2B} and α_{2C} AR; Link *et al*., 1996) mutation in the gene that encodes the α_2 AR. The D79N point mutation in the α_{2A} AR mutant results in selective uncoupling of the α_2 AR from activation of potassium currents; the coupling to inhibition of voltage-gated calcium channels and adenylate cyclase is not altered (Surprenant *et al*., 1992). However, although some of the signalling pathways are preserved in the α_{2A} AR D79N mutant mice, the density of α_{2A} AR binding in the mutant is reduced by 80% compared to wild-type mice (MacMillan *et al*., 1996).

Finally, because several studies have demonstrated that α_2 AR agonists produce antinociceptive effects in rodent models of acute pain (Reddy *et al*., 1980; Yaksh, 1985; Kalso *et al*., 1991; Takano & Yaksh, 1992) and of neuropathic pain (Yaksh *et al*., 1995, but see also Puke *et al*., 1994), and also in acute and chronic pain in humans (Tamsen & Gordh, 1984; Eisenach *et al*., 1996), we examined the contribution of the different α_2 AR subtypes to the antinociceptive effects of the α_2 AR agonist, dexmedetomidine. Previous studies suggested that the α_{2A} subtype mediates the antinociceptive effect of α_2 AR agonists in acute models of pain (Hunter *et al*., 1997; Lakhlani *et al*., 1997; Stone *et al*., 1997). However, because α_2 AR expression may be altered after nerve injury (Cho *et al*., 1997; Fareed *et al*., 1997; Birder & Perl, 1999; Stone *et al*., 1999; Shi *et al*., 2000), it is possible that the action of dexmedetomidine is altered by nerve injury or that the α_2 AR receptor subtype that is responsible for its antinociceptive effect is different.

Methods

Animals

The generation of the mutant mice has been described previously (MacMillan *et al*., 1996; Link *et al*., 1996). In the α_{2A} AR mutant mice, the receptor carries a D79N point mutation that uncouples the α_{2A} AR from potassium channels (MacMillan *et al*., 1996); these mice were obtained from Dr Lee Limbird (Vanderbilt University). By contrast, the α_{2B} AR and α_{2C} AR mutant mice carry a null mutation in the gene that encodes the α_2 AR (Link *et al*., 1996); these mice were obtained from Dr Brian Kobilka (Stanford University). The experiments were performed on age, weight (20–30 grams) and sex-matched mice. In the first experiment, which examined the effect of nerve injury in α_{2A} AR mutant and wild-type mice, male mice were used. In subsequent studies, female α_{2A} AR mutant and wild-type mice were used. We did not observe any difference between male and female mice in general behaviour or with respect to nerve injury-induced allodynia. Female mice were used for all experiments involving α_{2B} and α_{2C} AR mutant and wild-type mice. Because the D79N mice were generated on a 129SvJ background, we used 129SvJ wild-type mice (Jackson Laboratories) as controls. The mice that carried a null mutation in either the α_{2B} AR (129SvJ/C57BL) or α_{2C} AR (129SvJ/FVB) gene were on mixed genetic backgrounds (Link *et al*., 1996). Wild-type and homozygous mice were bred from heterozygous mice. Finally, the antagonist experiments were performed on male C57BL/6 mice weighing 20–25 g (Bantin-Kingman). All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco.

Nerve injury model

The nerve injury model was induced as we described previously (Malmberg & Basbaum, 1998). Briefly, we anaesthetized the mice using a mixture of ketamine (intraperitoneal (i.p.) injection of 66 mg kg^{-1} Ketalar®, Parke-Davis, NJ, U.S.A.) and Xylazine (13 mg kg^{-1}, i.p., Xylazine, Ben Venue Laboratories, OH, U.S.A.), and tied a tight ligature using 9-0 silk suture around approximately $\frac{1}{3}$ to $\frac{1}{2}$ the diameter of the sciatic nerve, similar to the approach described in rats by Seltzer *et al*. (1990). Before and at several times after the injury, we determined both the thermal and mechanical sensitivity of the hindpaw on the injured and uninjured side. Thermal sensitivity was assessed by measuring the paw withdrawal latency to a radiant heat stimulus (Hargreaves *et al*., 1988). Paw withdrawal latency was determined by three measurements per paw over a testing period of 30 min. We adjusted the stimulus intensity to yield a 10 s withdrawal latency in the normal mouse; cut off in the absence of a response was 20 s. Mechanical sensitivity was assessed with calibrated von Frey filaments using the up–down paradigm (Chaplan *et al*., 1994). As we previously described, we began the testing with the 0.3 g filament; cut off in the absence of a response was 2.5 g (Malmberg & Basbaum, 1998).

Guanethidine sympathectomy

Systemic delivery of guanethidine produces a functional sympathectomy by depleting noradrenaline from sympathetic terminals (Boullin *et al*., 1966; Johnson *et al*., 1975; Maxwell *et al*., 1960). In a previous study, we showed that a single injection of guanethidine produces a partial and reversible reduction of thermal and mechanical allodynia in C57BL/6 mice (Malmberg & Basbaum, 1998). Based on the experiments in our previous studies, in the present study we made a single i.p. injection of 50 mg kg^{-1} guanethidine, 8–10 days after the nerve injury. We evaluated the thermal and mechanical thresholds before and 1 day after the guanethedine injection.

Dexmedetomidine studies

To study the effect of α_2 AR activation, we used the α_2 AR agonist dexmedetomidine (synthesized at Roche Bioscience, Palo, Alto, CA, U.S.A.). After determining the nerve injury-induced thresholds at the time of maximal allodynia, 10–14

days after injury, the mice were randomly assigned to different groups and were given a subcutaneous (s.c.) injection of 3, 10 or 30 μg kg^{-1} dexmedetomidine or 10 ml kg^{-1} saline as control. Twenty to 40 min later, at the peak effect of dexmedetomidine, we re-tested the thermal and mechanical sensitivity of the mice. Because the effects of dexmedetomidine were reversible, we used the same group of mice to generate the dose-response curve; the mice were used no more than 2–3 times to examine the effects of dexmedetomidine. Two to 4 days after the first dexmedetomidine injection, the mice were again randomly assigned to different groups and the effect of 3, 10, 30 μg kg^{-1} dexmedetomidine or saline was examined. Five to six mice were tested at each dose of dexmedetomidine.

Antagonist studies

To determine the site of action of the antinociceptive effect of dexmedetomidine, we used the non-selective α_2 AR antagonist RS 42206 ([8aR-(8aα,12aα,13aα)]-N-[3-[5, 8a,9, 10,11, 12a,13a-octahydro-3-methoxy-6H-isoquino[2,1-g]naphthyridin-12 (8H) -yl) sulphonyl] -methanesulphonamide-HCl, synthesized at Roche Bioscience, Palo, Alto, CA, U.S.A., Clark *et al*., 1993). RS 42206 has been shown to be peripherally selective with a 47 fold ratio between its antagonistic effect of mydriasis in anaesthetized rats (central activity) or pressor effects in pithed rats (peripheral activity) after a single injection (M. Spedding and R.D. Clark, unpublished observations. For testing of similar compounds and methods, see Clark *et al*., 1991 and Xiao & Rand, 1990). The selectivity is believed to reflect a lack of central penetration by RS 42206. The α_2 AR affinity of RS 42206 was determined as previously described (Jasper *et al*., 1998). Radioligand binding affinity estimates (pK_i at human α_{2A}-, α_{2B}- and α_{2C} AR for RS 42206 were 8.4, 8.3 and 8.0, respectively.

To inhibit peripheral α_2 AR we delivered RS 42206 i.p. In a separate group of mice, we administered RS 42206 intrathecally to selectively block spinal α_2 AR. Intrathecal delivery of RS 42206 was made by direct lumbar puncture (Hylden & Wilcox, 1980) in a volume of 5.0 μl. The systemic dose of 0.3 mg kg^{-1} RS 42206 i.p. was based on preliminary experiments (Hunter *et al*., unpublished observations) and the intrathecal dose of 30 μg was based on preliminary dose-response experiments (Malmberg & Basbaum, unpublished observations). The antagonist was administered 10 min before the injection of dexmedetomidine and the sensitivity to thermal and mechanical stimulation was assessed 20–40 min later.

Quantification and statistical analyses

The behavioural data are presented as mean$\pm$s.e.mean at different time points after the nerve injury. For statistical analysis of differences in thermal sensitivity, we used repeated measures analysis of variance (ANOVA) followed by the Student Newman-Keuls test for multiple comparisons. Statistical comparisons of mechanical thresholds were carried out using the non-parametric Friedman test followed by the Dunn's test for multiple comparisons (see Chaplan *et al*., 1994, for choice of statistical analysis of changes in mechanical thresholds). Statistical analysis of changes in thermal or mechanical sensitivity after drug treatment (e.g., guanethidine, dexmedetomidine or RS 42206) was carried out using paired *t*-test (thermal) or Wilcoxon signed rank test (mechanical), respectively. We compared post-guanethidine or post-dexmedetomidine latencies or thresholds to the values before the injection, but after the nerve injury.

Results

Effects of nerve injury

As previously described (Macmillan *et al*., 1996; Link *et al*., 1996), all the mutant mice showed normal general behaviour. Thermal response latencies and mechanical withdrawal thresholds before the nerve injury did not differ between the mutant and wild-type mice in any of the different groups. We also did not observe any significant difference in the development of thermal or mechanical allodynia in the α_{2A}, α_{2B} and α_{2C} AR mutant wild-type mice (Figures 1 and 2). In

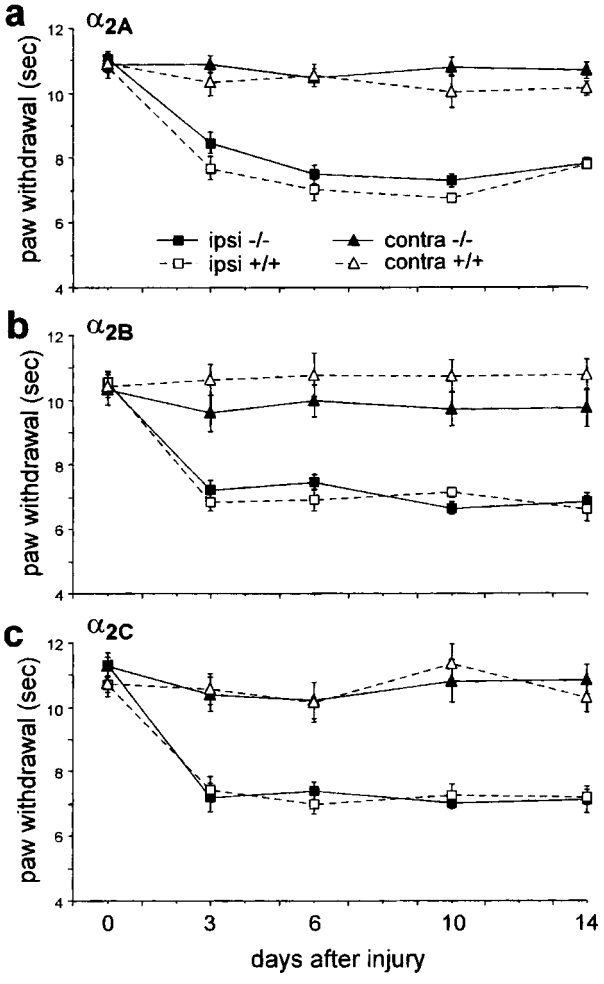

Figure 1 Effect of nerve injury in (a) α_{2A}, (b) α_{2B} and (c) α_{2C} AR mutant (−/−) and wild-type (+/+) mice on thermal response latencies of the injured (ipsi) and the non-injured (contra) paw. Nerve injury produced comparable thermal allodynia in the wild-type and mutant mice. Data are presented as the mean$\pm$s.e.mean; $n=10$ per group.

all groups of mice we observed an increased sensitivity to thermal (Figure 1) and mechanical (Figure 2) stimulation of the hindlimb ipsilateral to the partial nerve injury. Although there was some variability among groups in the response thresholds of the hindlimb contralateral side to the nerve injury (data not shown), the contralateral changes were not statistically different.

Effects of guanethidine sympathectomy

We only examined the effect of guanethidine in mice that showed a reduced thermal withdrawal latency of at least 20% and a decrease of at least 50% in the mechanical withdrawal threshold. In our experiments approximately 75–80% of nerve injured mice met these criteria. Intraperitoneal injection of 50 mg kg^{-1} of guanethidine produced a partial relief of thermal and mechanical allodynia 24 h post-administration in the different groups of mice (Figures 3 and 4). For some groups the change was significant (thermal latencies for α_{2A} wild-type and mutant, α_{2B} wild-type, α_{2C} wild-type and mutant, and mechanical thresholds for α_{2A} wild-type, α_{2C} wild-type, α_{2C} mutant mice) whereas other groups showed non-significant changes (e.g., thermal thresholds for α_{2B} mutant, and mechanical thresholds for α_{2A} mutant, α_{2B} wild-type and mutant mice). However, the effect was in general small, particularly on the mechanical allodynia (Figure 4). Compared to pre-injury thresholds (indicated with a dotted line in the graph), significant thermal ($P<0.05$, paired t-test) and mechanical allodynia ($P<0.05$, Wilcoxon signed rank test) persisted for all the groups after the

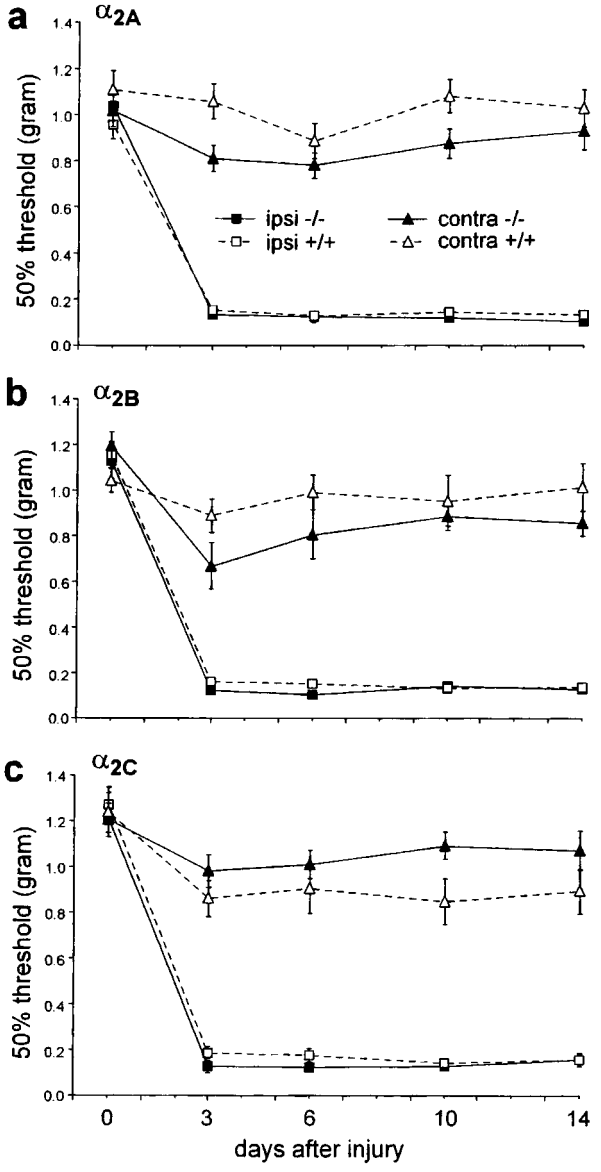

Figure 2 Effect of nerve injury in (a) α_{2A}, (b) α_{2B} and (c) α_{2C} AR mutant (−/−) and wild-type (+/+) mice on mechanical paw withdrawal thresholds of the injured (ipsi) and the non-injured (contra) paw. Nerve injury produced comparable mechanical allodynia in the wild-type and mutant mice. Data are presented as the mean±s.e.mean; $n=10$ per group.

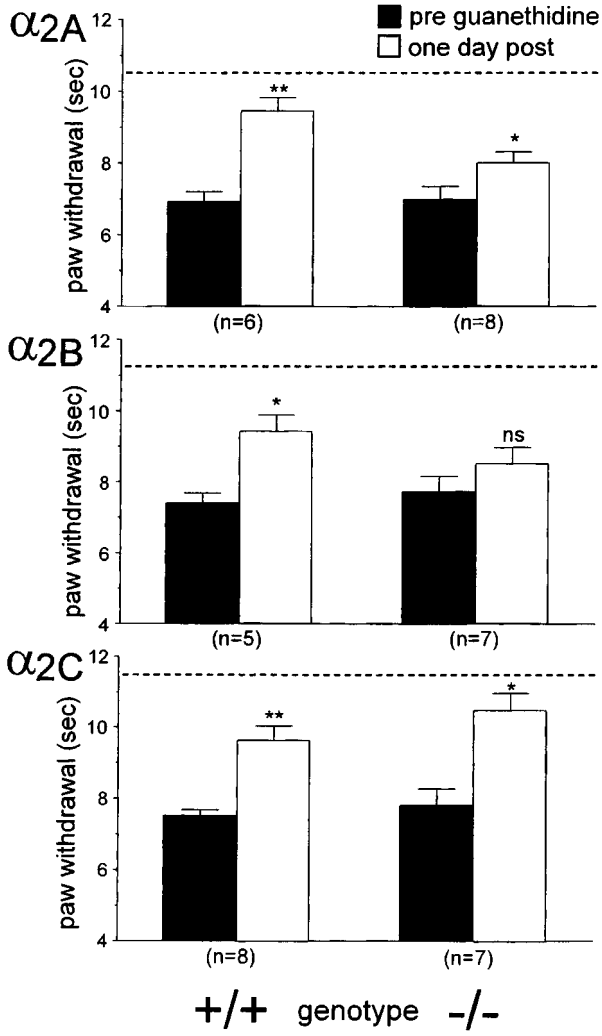

Figure 3 Effect of 50 mg kg^{-1} guanethidine on thermal allodynia of the injured paw in (a) α_{2A}, (b) α_{2B} and (c) α_{2C} AR mutant (−/−) and wild-type (+/+) mice. Thermal paw withdrawal latencies were determined before (PRE) and 24 h after the injection of guanethidine (POST). Data are presented as the mean±s.e.mean. The asterisks indicate that guanethidine produced a significant increase in paw withdrawal latencies (comparing PRE vs PST, paired t-test; $^*P<0.05$, $^{**}P<0.01$). The dotted line indicates the baseline latency before the nerve-injury surgery was performed.

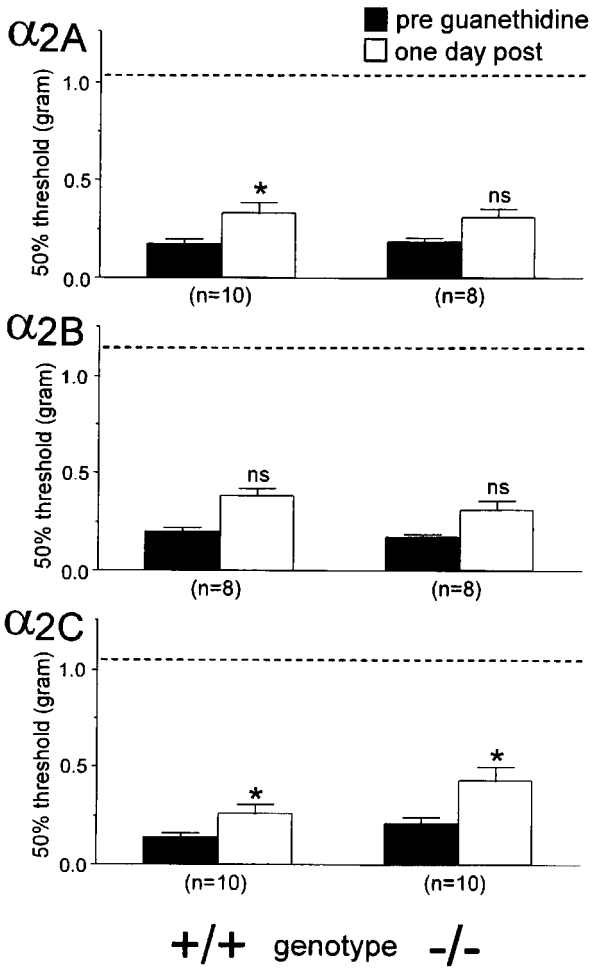

Figure 4 Effect of 50 mg kg^{-1} guanethidine on mechanial allodynia of the injured paw in (a) α_{2A}, (b) α_{2B} and (c) α_{2C} AR mutant ($-/-$) and wild-type ($+/+$) mice. Mechanical thresholds were determined before (PRE) and 24 h after the injection of guanethedine (POST). Data are presented as the mean$\pm$s.e.mean. The asterisks indicate a significant decrease in mechanical sensitivity (Wilcoxon signed rank test; $*P<0.05$) comparing PRE and POST. The dotted line indicates the baseline latency before the nerve injury was performed.

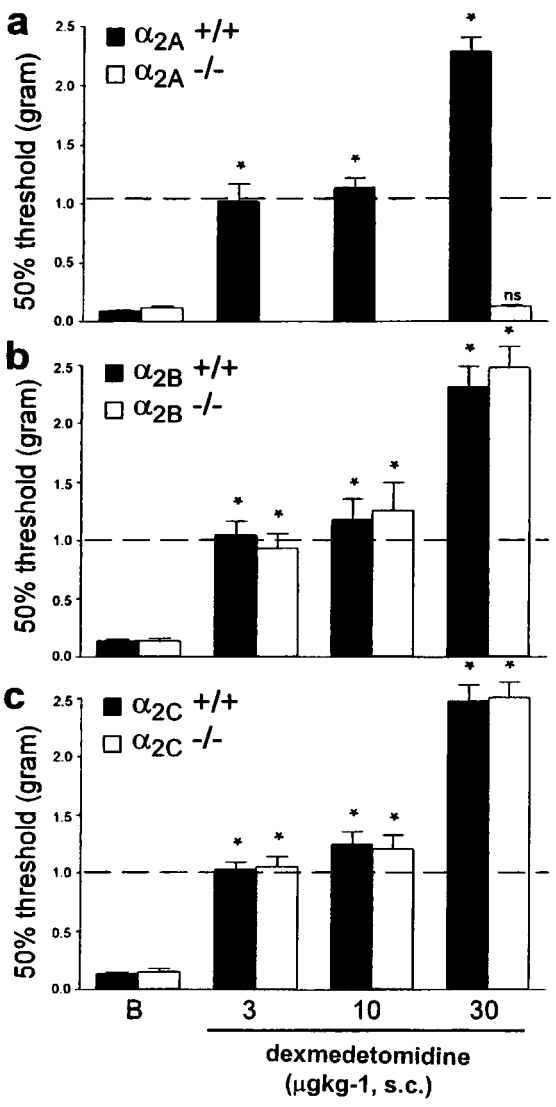

Figure 5 Effect of dexmedetomidine on thermal allodynia of the injured paw in (a) α_{2A}, (b) α_{2B} and (c) α_{2C} AR mutant and wild-type mice. Ten to 14 days after the nerve transection, we established post-injury baseline (b) latencies. The animals then received a s.c. injection of dexmedetomidine and 20–40 min later we again measured thermal sensitivity. The asterisks indicate significant increases in paw withdrawal latencies ($***P<0.001$, ANOVA followed by Student Neuman-Keuls *post hoc* test) compared to post-injury baseline (indicated by 'B' in the figure) values. The dotted line indicates the 'normal' paw withdrawal latencies before the nerve injury was performed; an increase in latency above this value is considered to be antinociceptive. Data are presented as the mean$\pm$s.e.mean; $n=5-6$ per dose.

guanethidine treatment (the pre-injury data are not shown, although these thresholds are indicated by a dotted line in Figures 3 and 4).

Effects of dexmedetomidine

Dexmedetomidine dose-dependently increased thermal latencies and mechanical thresholds in α_{2A} wild-type, α_{2B} and α_{2C} AR mutant and wild-type mice (Figures 5 and 6). By contrast, in the α_{2A} AR mutant mice, dexmedetomidine was without effect, even at a dose of 100 μg kg^{-1}, which in α_{2A} AR wild-type mice produces significant side effects, including sedation and motor dysfunction (data not shown). Since 30 μg kg^{-1} dexmedetomidine was ineffective in the α_{2A} AR mutant mice, we did not try lower doses in those animals (Figures 5 and 6). At the lowest effective dose (3.0 mg kg^{-1}, s.c.), dexmedetomidine exerted an anti-allodynic effect in α_{2A} wild-type, and in α_{2B} and α_{2C} AR mutant and wild-type mice, i.e., it returned the thermal and mechanical thresholds to pre-injury levels (Figures 5 and 6). This dose did not affect withdrawal thresholds on the non-injured side (data not shown), whereas at higher doses (10–30 μg kg^{-1}), dexmedetomidine produced an antinociceptive/analgesic effect as indicated by increased paw withdrawal latencies and thresholds to thermal (Figure 5) and mechanical (Figure 6) stimulation, respectively, on both the injured and the uninjured sides.

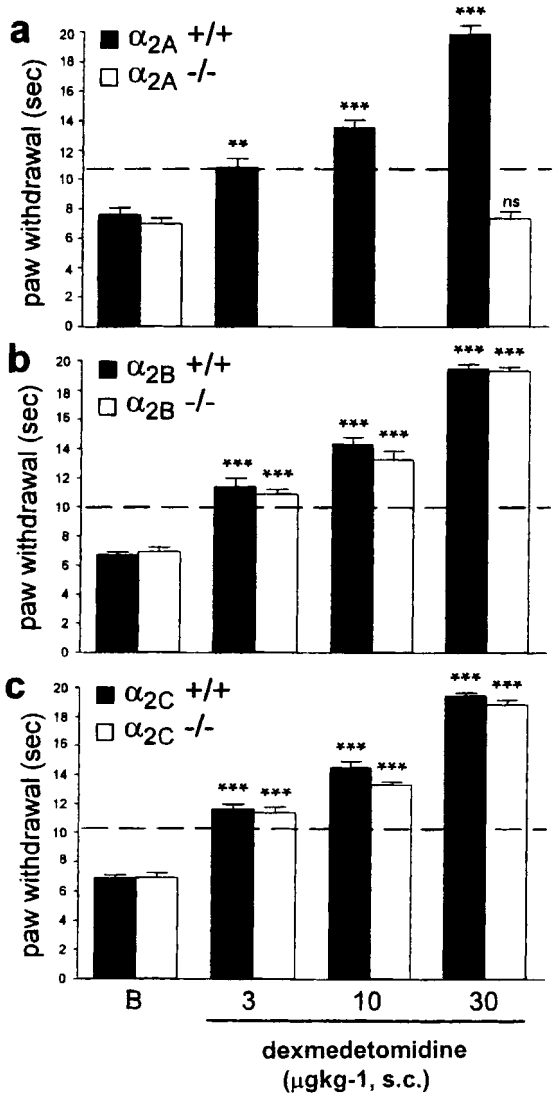

Figure 6 Effect of dexmedetomidine on mechanical allodynia of the injured paw in (a) α_{2A}, (b) α_{2B} and (c) α_{2C} AR mutant and wild-type mice. Ten to 14 days after the nerve injury, we established post-injury baseline (b) mechanical withdrawal thresholds. The animals then received a s.c. injection of dexmedetomidine and 20–40 min later we again measured mechanical thresholds. The asterisks indicate significant increases in paw withdrawal thresholds ($^*P<0.05$, Friedman test followed by Wilcoxon signed rank test) compared to post-injury baseline (indicated by 'B' in the figure) values. The dotted line indicates mechanical thresholds of the paw before the nerve injury was performed. Data are presented as the mean±s.e.mean; $n=5-6$ per dose.

Antagonist studies

Antagonism of peripheral α_2 AR by the i.p. injection of 0.3 mg kg^{-1} RS 42206 did not change the thermal or mechanical thresholds in nerve-injured mice (data not shown). Furthermore, pre-treatment with 0.3 mg kg^{-1} RS 42206 before the administration of an anti-allodynic dose of dexmedetomidine (3 μg kg^{-1}, s.c.) was ineffective at antagonizing the effect of dexmedetomidine (Figure 7). Similarly, systemic injection of 0.3 mg kg^{-1} RS 42206 did not affect the antinociceptive action of 30 μg kg^{-1} dexmedetomidine (Figure 8). By contrast, intrathecal administration of 30 μg RS 42206 completed blocked the effect of 30 μg kg^{-1} dexmedetomidine, implicating a spinal site for the antinociceptive effect of dexmedetomidine (Figure 9).

Discussion

In the present study, mutant mice that carry either a point (α_{2A}) or null (α_{2B} and α_{2C}) mutation in the gene that encodes the α_2 AR were used to examine the contribution of α_2 AR subtypes to indices of neuropathic pain (thermal and mechanical allodynia) and to the antinociception produced by central α_2 AR activation. We found that the α_{2A}, α_{2B} and α_{2C} AR mutant mice developed a thermal and mechanical allodynia after peripheral nerve injury that was comparable to those observed in wild-type mice. These studies suggest that none of the α_2 AR receptor subtypes, by themselves, are required for the development of these indices of neuropathic pain. We also assessed the contribution of the sympathetic nervous system to the allodynia produced by nerve injury in the different α_2 AR mutant mice. Because guanethidine sympathectomy was only modestly effective in all groups of mice, factors other than the sympathetic nervous system can sustain the allodynia produced by this model of nerve injury. Furthermore, we demonstrated that dexmedetomidine produced an anti-allodynic effect at low doses and an antinociceptive effect at higher dose in all but the α_{2A} AR mutant mice. This observation strongly suggests that the

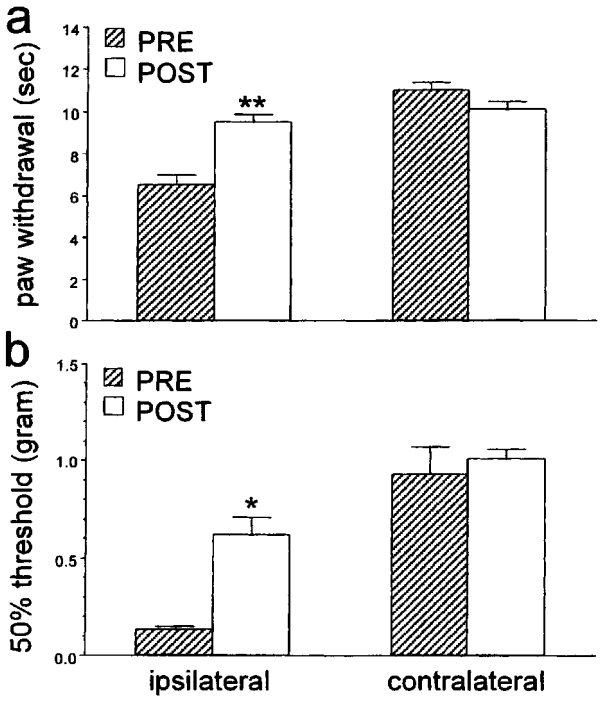

Figure 7 The effect of RS 42206 (0.3 mg kg^{-1}, i.p.) on all anti-allodynic dose of dexmedetomidine (3.0 μg kg^{-1}, s.c.) in nerve injured mice. Data are presented as the mean±s.e.mean for (a) thermal paw withdrawal latency and (b) mechanical paw withdrawal thresholds of the injured (ipsi) and the non-injured (contra) side. $N=6$ per group. The asterisks indicate significant increases (a: $^{**}P<0.01$, t-test, b: $^*P<0.05$, Wilcoxon signed rank test) comparing latencies or thresholds before and after drug treatment.

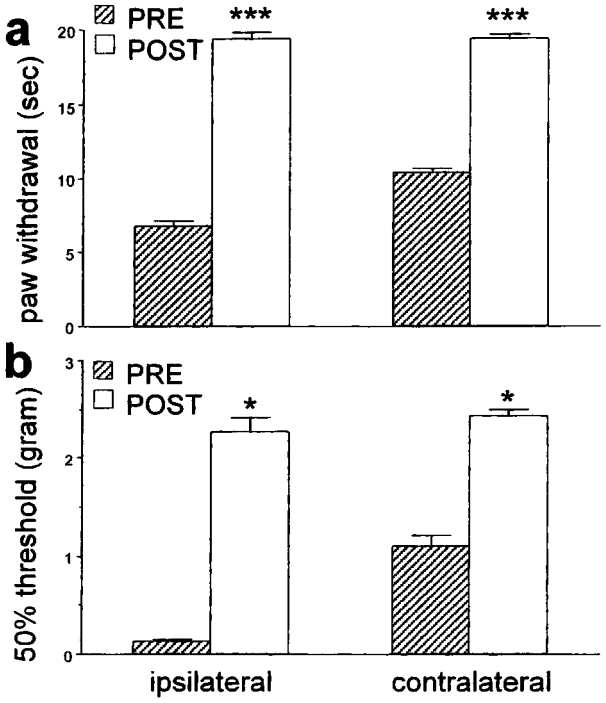

Figure 8 The effect of RS 42206 (0.3 mg kg^{-1}, i.p.) on an antinociceptive dose of dexmedetomidine (30 μg kg^{-1}, s.c.) in nerve-injured mice. Data are presented as the mean$\pm$s.e.mean for (a) thermal paw withdrawal latency and (b) mechanical paw withdrawal thresholds of the injured (ipsi) and the non-injured (contra) paws. $N=6$ per group. The asterisks indicate significant increases (a: ***$P<0.001$, t-test, b: *$P<0.05$, Wilcoxon signed rank test) comparing latencies or thresholds before and after drug treatment.

pain-relieving effects of dexmedetomidine are indeed mediated *via* the α_{2A} AR. Furthermore, using the hydrophilic α_2 AR antagonist RS 42206 to selectively block the peripheral or central α_2 AR, we showed that both the anti-allodynic and antinociceptive effects of dexmedetomidine are likely modulated by an action at the spinal cord level.

The development of genetically altered mice with a selective mutation in the genes encoding the different α_2 AR subtypes has provided powerful tools to examine the contribution of these receptors to various physiological functions, including nociception. On general behavioural grounds, the mutant and wild-type mice cannot be distinguished. Of the studies previously published using the different α_2 AR mutant mice, none report compensatory changes or abnormal behaviour due to the mutation (MacMillan *et al*., 1996; Link *et al*., 1996; Hunter *et al*., 1997; Stone *et al*., 1997). In contrast to the α_{2B} and α_{2C} AR mutant mice, which lack the receptor protein due to a null mutation (Link *et al*., 1996), the α_{2A} AR mutant mice express a point (D79N) mutation in this receptor (MacMillan *et al*., 1996). The D79N mutation selectively uncouples the receptor from activation of potassium currents, but retains coupling to inhibition of voltage-gated calcium channels and cyclic AMP production (Surprenant *et al*., 1992). As described above, although the calcium and adenylate cyclase signalling properties are presumably intact in the α_{2A} AR D79N mouse, the density of α_{2A} AR in this mouse is reduced by 80% compared to wild-type mice (MacMillan *et al*., 1996). For this reason we cannot exclude the possibility that the residual receptor, which retains coupling to voltage-gated calcium channels and adenylate cyclase, is sufficient to contribute to the development of neuropathic pain in these mice. However, we believe that this is unlikely because we also showed that blocking all peripheral α_2 AR subtypes with RS 42206 had no effect on neuropathic pain behaviour. This provides further support for the proposal that α_2 AR are not required for the development of neuropathic pain in this model.

Several previous reports indicated an involvement of α_2 AR in the sympathetic-sensory coupling after nerve injury (Tracey *et al*., 1995; Xie *et al*., 1995; Chen *et al*., 1996). The observation that sympathectomy produced either no or, at best, a partial relief of the allodynia was, therefore, somewhat unexpected. This result was, however, consistent with the fact that we did not find a difference in nerve injury-induced pain between the different α_2 AR mutant and wild-type mice. In fact, there is considerable disagreement as to the effect of sympatholytic therapy on the pain behaviour produced by various peripheral nerve injuries. Some studies have reported beneficial effects (Neil *et al*., 1991; Shir & Seltzer, 1991; Kim & Chung, 1991; Kim *et al*., 1993; Desmeules *et al*., 1995) while others have concluded that the procedures provided no relief of the allodynia (Willenbring *et al*., 1995; Lavand'homme *et al*., 1998; Ringkamp *et al*., 1999b). The use of different neuropathic pain models may contribute to observed variability of sympathectomy on neuropathic pain behaviour (Kim *et al*., 1997).

In the present study we observed a considerably smaller anti-allodynic effect of guanethidine compared to our previous study (Malmberg & Basbaum, 1998). The difference may be related, in part, to the different strains used in the two studies (C57/BL6 in the first study and 129Sv/J and 129SvJ/C57BL or 129SvJ/FVB mixed genetic backgrounds in the present study) and/or different sex (female) used in the two studies. Thus, recently it was demonstrated that different strains of rats show variability with regard to adrenergic sensitivity of the neuropathic pain behaviour (Lee *et al*., 1997). However, the observed partial effect of guanethidine and the development of neuropathic pain after nerve injury in the α_2 AR subtype selective mutant mice, may indicate that the adrenergic nervous system contribution to the neuropathic pain behaviour is mediated *via* a different receptor than the α_2 AR. Indeed, studies in the primate (Ali *et al*., 1999) and in patients (Davis *et al*., 1991; Drummond *et al*., 1996) indicate that the α_1 AR is a critical determinant of the adrenergic sensitivity after peripheral nerve injury.

In the present study, we found that systemic delivery of the α_2 AR agonist dexmedetomidine blocked the thermal and mechanical allodynia produced by nerve injury. Several studies have previously shown that activation of α_2 AR by dexmedetomidine produces analgesia in both acute and persistent pain models in rodents (Kalso *et al*., 1991; Fisher *et al*., 1991; Takano & Yaksh, 1992; Yaksh *et al*., 1995). At higher doses, dexmedetomidine produced an effect on thermal latencies and mechanical thresholds on both the injured and non-injured hindlimbs, indicating that dexmedetomidine mediated an antinociceptive/analgesic effect in nerve-injured mice. In contrast, a lower dose of dexmedetomidine reversed the allodynia ipsilateral to the nerve injury, but had no effect on the non-injured (contralateral) side. Thus, at low doses

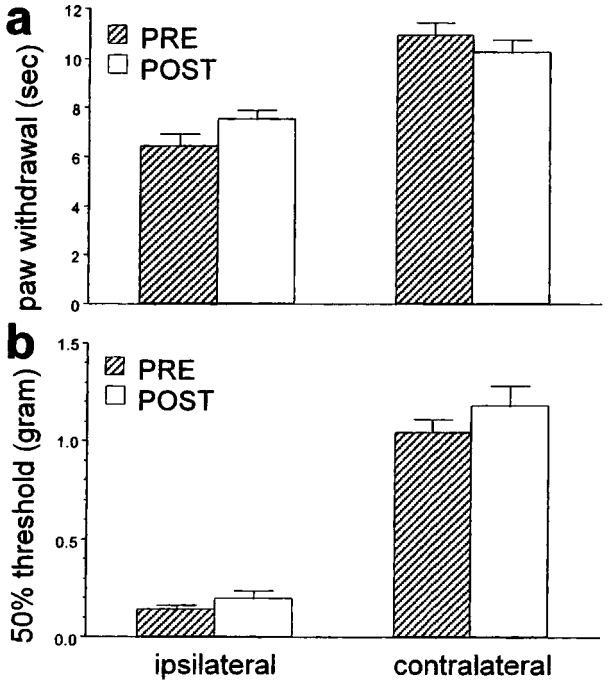

Figure 9 The effect of intrathecal injection of 30 μg RS 42206 on an analgesic dose of dexmedetomidine (30 μg kg^{-1}, s.c.) in nerve-injured mice. Data are presented as the mean$\pm$s.e.mean (a) thermal paw withdrawal latency and (b) mechanical paw withdrawal thresholds of the inured (ipsi) and the non-injured (contra) paws. There was no significant difference (a: $P>0.05$, *t*-test, b: $P>0.05$, Wilcoxon signed rank test) between pre and post thermal latencies or mechanical thresholds indicating that RS 42206 reversed the effect of dexmedetomidine. $N=6$ per group.

dexmedetomidine is exclusively an anti-allodynic agent. The observation that dexmedetomidine was active on the injured side at a dose that did not affect the contralateral limb, further suggests that the injury induces adrenergic sensitivity. This may occur at the level of the ipsilateral dorsal root ganglia (DRG) and/or the spinal dorsal horn, where changes in α_2 AR expression have been described (Cho *et al*., 1997; Fareed *et al*., 1997; Birder & Perl, 1999; Stone *et al*., 1999; Shi *et al*., 2000). However, the direction of the change is unclear. One report indicated that α_{2A} AR immunoreactivity was upregulated in DRG neurons after sciatic nerve injury (Birder & Perl, 1999), but another study found evidence for downregulation in the spinal dorsal horn after nerve injury (Stone *et al*., 1999). Moreover, while two studies showed that mRNA for α_{2A} AR was reduced after spinal nerve injury (Fareed *et al*., 1997; Cho *et al*., 1997), a recent study demonstrated an increase of this subtype in DRG neurons after nerve transection (Shi *et al*., 2000). Nerve injury has been shown to produce a decrease in α_{2C} AR mRNA in DRG neurons (Cho *et al*., 1997), while α_{2C} AR immunoreactivity was unchanged or increased at the spinal cord level using a similar spinal nerve-injury model (Stone *et al*., 1999).

Although the direction of the change in receptor expression after nerve injury is unclear, it is likely that an alteration of the α_{2A} and α_{2C} AR expression in the DRG and/or spinal cord underlies the shift in dexmedetomidine sensitivity that we observed in the nerve-injured mice. On the other hand, because changes in DRG receptor expression are usually mirrored by changes in the receptor expression at both the peripheral and central terminals of primary afferent fibres, it is possible that both central and peripheral sites are involved in the altered response to dexmedetomidine. By selectively antagonizing peripheral or central/spinal α_2 AR with RS 42206 we showed that both the anti-allodynic and the antinociceptive effects of dexmedetomidine are produced by interaction with α_2 AR in the spinal cord. Furthermore, we found that the anti-allodynic and the antinociceptive effects of dexmedetomidine were intact in α_{2B} and α_{2C} AR, but absent in the α_{2A} AR mutant mice. Our results agree with previous reports showing that the antinociceptive effect of dexmedetomidine in the hot-plate and tail-flick tests is mediated *via* an activation of α_{2A} AR (Lakhlani *et al*., 1997; Hunter *et al*., 1997; Stone *et al*., 1997) and indicate that the same receptor comes into play under both acute and persistent pain conditions.

Because the majority of α_{2A} AR in the spinal cord are located on peptide-containing primary afferents neurons that terminate in the dorsal horn (Stone *et al*., 1999), the antinociceptive action of dexmedetomidine may involve inhibition of peptide release at these terminals. In fact, activation of α_2 AR has been shown to reduce capsaicin, potassium and noxious stimulus-evoked release of substance P and calcitonin gene-related peptide from spinal cord slices or from cultured primary afferent neurons (Kuraishi *et al*., 1985; Pang & Vasko, 1986; Holz *et al*., 1989; Takano *et al*., 1993), probably by an action on N-type calcium channels (Cox & Dunlap, 1992). The antinociceptive effects of dexmedetomidine may, of course, also be mediated by postsynaptic inhibitory mechanisms, *via* α_2 AR that are expressed on nociresponsive projection neurons.

In summary, our studies indicate that none of the α_2 AR subtypes are essential for the development of a profound behavioural allodynia after peripheral nerve injury in a mouse model of neuropathic pain. Taken together with our previous results, however, it is possible that the extent to which the sympathetic nervous system contributes to the nerve injury-induced allodynia depends on the genetic background of the particular mouse that is studied. We also found that the antinociceptive effect of dexmedetomidine was abolished in the D79N α_{2A} AR mutant mice, which indicates that the α_{2A} AR is critical for the pain relieving actions of α_2 AR agonists in this mouse model of neuropathic pain. Consistent with previous findings, we demonstrated that the anti-allodynic and antinociceptive effects of dexmedetomidine can only be reversed by blocking spinal α_{2A} AR. The fact that an anti-allodynic effect could be produced at much lower doses than were required to produce frank antinociception is of particular interest. Specifically, it may be possible to achieve clinical efficacy in neuropathic pain conditions without the α_{2A} AR-mediated side effects, such as sedation and hypothermia (Hunter *et al*., 1997), that occur when higher doses are used.

We thank Dr Robin Clark and Michael Spedding for information about RS 42206 and Heather Gilbert for technical assistance. We also thank Dr Lee Limbird for providing the α_{2A} AR mutant mice and Dr Brian Kobilka for the aα_{2B} and α_{2C} AR null mutation mice. This work was supported by DE 08973 and NS 21445 (A.I. Basbaum) and the Pharmaceutical Research and Manufacturers of America Foundation (A.B. Malmberg).

References

ALI, Z., RINGKAMP, M., HARTKE, T.V., CHIEN, H.F., FLAVAHAN, N.A., CAMPBELL, J.N. & MEYER, R.A. (1999). Uninjured C-fiber nociceptors develop spontaneous activity and adrenergic sensitivity following L6 spinal nerve ligation in monkey. *J. Neurophysiol.,* **81,** 455 – 466.

ARNER, S. (1991). Intravenous phentolamine test: diagnostic and prognostic use in reflex sympathetic dystrophy. *Pain,* **46,** 17 – 22.

BIRDER, L.A. & PERL, E.R. (1999). Expression of alpha2-adrenergic receptors in rat primary afferent neurones after peripheral nerve injury or inflammation. *J. Physiol.,* **515,** 533 – 542.

BONICA, J.J. (1990). Causalgia and other reflex sympathetic dystrophies. In *The Management of Pain*. ed. Bonica, J.J. pp. 220 – 243. Philadelphia, PA: Lea and Febinger.

BOULLIN, D.J., COSTA, E. & BRODIE, B.B. (1966). Discharge of tritium-labeled guanethidine by sympathetic nerve stimulation as evidence that guanethidine is a false transmitter. *Life Sci.,* **5,** 803 – 808.

BYLUND, D.B., EIKENBERG, D.C., HIEBLE, J.P., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P., MOLINOFF, P.B., RUFFOLO, R.J. & TRENDLENBURG, U. (1994). International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.,* **46,** 121 – 136.

CHAPLAN, S.R., BACH, F.W., POGREL, J.W., CHUNG, J.M. & YAKSH, T.L. (1994). Quantitative assessment of tactile allodynia in the rat paw. *J. Neurosci. Meth.,* **53,** 55 – 63.

CHEN, Y., MICHAELIS, M., JÄNIG, W. & DEVOR, M. (1996). Adrenoceptor subtype mediating sympathetic-sensory coupling in injured sensory neurons. *J. Neurophysiol.,* **76,** 3721 – 3730.

CHO, H.J., KIM, D.S., LEE, N.H., KIM, J.K., LEE, K.M., HAN, K.S., KANG, Y.N. & KIM, K.J. (1997). Changes in the alpha 2-adrenergic receptor subtypes gene expression in rat dorsal root ganglion in an experimental model of neuropathic pain. *Neuroreport,* **8,** 3119 – 3122.

CLARK, R.D., REPKE, D.B., BERGER, J., NELSON, J.T., KILPATRICK, A.T., BROWN, C.M., MACKINNON, A.C., CLAGUE, R.U. & SPEDDING, M. (1991). Structure-affinity relationships of 12-sulfonyl derivatives of 5,8,8A,9,10,11,12,12a,13,13a-decahydro-6h-isoquino[2,1-g][1,6] naphthyridines at alpha-adrenoceptors. *J. Med. Chem.,* **34,** 705 – 717.

CLARK, R.D., SPEDDING, M. & REDFERN, W.S. (1993). Preparation of decahydro-8H-isoquino[2,1-g][1,6]naphthyridine and decahydrobenzo[a]pyrrolo[3,2-q]quinolizine derivatives as α2-adrenoceptor antagonist. (Syntex (USA), Inc., USA). Eur. Pat. Appl., 69 pp. CODEN: EPXXDW EP 524004 A1 19930120, Application: EP 92-306553 19920716.

COX, D.H. & DUNLAP, K. (1992). Pharmacological discrimination of N-type from L-type calcium current and its selective modulation by transmitters. *J. Neurosci.,* **12,** 906 – 914.

DAVIS, K.D., TREEDE, R.D., RAJA, S.N., MEYER, R.A. & CAMPBELL, J.N. (1991). Topical application of clonidine relieves hyperalgesia in patients with sympathetically maintained pain. *Pain,* **47,** 309 – 317.

DESMEULES, J.A., KAYSER, V., WEIL-FUGGAZA, J., BERTRAND, A. & GUILBAUD, G. (1995). Influence of the sympathetic nervous system in the development of abnormal pain-related behaviours in a rat model of neuropathic pain. *Neuroscience,* **67,** 941 – 951.

DEVOR, M., JÄNIG, W. & MICHAELIS, M. (1994). Modulation of activity in dorsal root ganglion neurons by sympathetic activation in nerve-injured rats. *J. Neurophysiol.,* **71,** 38 – 47.

DEVOR, M. & JÄNIG, W. (1981). Activation of myelinated afferents ending in a neuroma by stimulation of the sympathetic supply in the rat. *Neurosci. Lett.,* **24,** 43 – 47.

DRUMMOND, P.D., SKIPWORTH, S. & FINCH, P.M. (1996). Alpha 1-adrenoceptors in normal and hyperalgesic human skin. *Clin. Sci.,* **91,** 73 – 77.

EISENACH, J.C., DEKOCK, M. & KLIMSCHA, W. (1996). Alpha-2 adrenergic agonists for regional anesthesia. *Anesthesiology,* **85,** 655 – 674.

FAREED, M.U., NOH, H.R., CHUNG, J.M. & DAVAR, G. (1997). Identification and increased expression of a novel alpha-2 adrenoceptor mRNA in a rat model of experimental painful neuropathy. In *Proc of the 8th World Congress on Pain, Progress in Pain Research and Management*. eds. Jensen, T.S., Turner, J.A., Wiesenfeld-Hallin, Z. Vol. 8, pp. 539 – 546, Seattle: IASP Press.

FISHER, B., ZORNOW, M.H., YAKSH, T.L. & PETERSON, B.M. (1991). Antinociceptive properties of intrathecal dexmedetomidine in rats. *Eur. J. Pharmacol.,* **192,** 221 – 225.

GOLD, M.S., DASTMALCHI, S. & LEVINE, J.D. (1997). Alpha 2-adrenergic receptor subtypes in rat dorsal root and superior cervical ganglion neurons. *Pain,* **69,** 179 – 190.

HARGREAVES, K., DUBNER, R., BROWN, F., FLORES, C. & JORIS, J. (1988). A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain,* **32,** 141 – 167.

HOLZ, IV, G.G., KREAM, R.M., SPIEGEL, A. & DUNLAP, K. (1989). G proteins couple alpha-adrenergic and GABAb receptors to inhibition of peptide secretion from peripheral sensory neurons. *J. Neurosci.,* **9,** 657 – 666.

HUNTER, J.C., FONTANA, D.J., HEDLEY, L.R., JASPER, J.R., LEWIS, R., LINK, R.E., SECCHI, R., SUTTON, J. & EGLEN, R.M. (1997). Assessment of the role of alpha2-adrenoceptor subtypes in the antinociceptive, sedative and hypothermic action of dexmedetomidine in transgenic mice. *Br. J. Pharmacol.,* **122,** 1339 – 1344.

HYLDEN, J.L.K. & WILCOX, G.L. (1980). Intrathecal morphine in mice: a new technique. *Eur. J. Pharmacol.,* **67,** 313 – 316.

JASPER, J.R., LESNICK, J.D., CHANG, L.K., YAMANISHI, S.S., CHANG, T.K., HSU, S.A., DAUNT, D.A., BONHAUS, D.W. & EGLEN, R.M. (1998). Ligand efficacy and potency at recombinant alpha-2 adrenergic receptors: agonist-mediated [^{35}S]GTPgamaS binding. *Biochem. Pharmacol.,* **55,** 1035 – 1043.

JÄNIG, W., LEVINE, J.D. & MICHAELIS, M. (1996). Interactions of sympathetic and primary afferent neurons following nerve injury and tissue trauma. *Prog. Brain Res.,* **113,** 161 – 184.

JOHNSON JR., E.M., CANTOR, E. & DOUGLAS JR., J.R. (1975). Biochemical and functional evaluation of the sympathectomy produced by the administration of guanethidine to newborn rat. *J. Pharmacol. Exp. Ther.,* **193,** 503 – 512.

KALSO, E.A., POYHIA, R. & ROSENBERG, P.H. (1991). Spinal antinociception by dexmedetomidine, a highly selective alpha 2-adrenergic agonist. *Pharmacol. Toxicol.,* **68,** 140 – 143.

KIM, K.J., YOON, Y.W. & CHUNG, J.M. (1997). Comparison of three rodent neuropathic pain models. *Exp. Brain Res.,* **113,** 200 – 206.

KIM, S.H. & CHUNG, J.M. (1991). Sympathectomy alleviates mechanical allodynia in an experimental animal model for neuropathy in the rat. *Neurosci. Lett.,* **134,** 131 – 134.

KIM, S.H., NA, H.S., SHEEN, K. & CHUNG, J.M. (1993). Effects of sympathectomy on a rat model of peripheral neuropathy. *Pain,* **55,** 85 – 92.

KURAISHI, Y., HIROTA, N., SATO, Y., KANEKO, S., SATOH, M. & TAKAGI, H. (1985). Noradrenergic inhibition of the release of substance P from the primary afferents in rabbit spinal dorsal horn. *Brain Res.,* **359,** 177 – 182.

LAKHLANI, P.P., MACMILLAN, L.B., GUO, T.Z., MCCOOL, B.A., LOVINGER, D.M., MAZE, M. & LIMBIRD, L.E. (1997). Substitution of a mutant alpha2a-adrenergic receptor via 'hit and run' gene targeting reveals the role of this subtype in sedative, analgesic, and anesthetic-sparing responses in vivo. *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 9950 – 9955.

LAVAND'HOMME, P., PAN, H.L. & EISENACH, J.C. (1998). Intrathecal neostigmine, but not sympathectomy, relieves mechanical allodynia in a rat model of neuropathic pain. *Anesthesiology,* **89,** 493 – 499.

LEE, D.H., CHUNG, K. & CHUNG, J.M. (1997). Strain differences in adrenergic sensitivity of neuropathic pain behaviors in an experimental rat model. *Neuroreport,* **8,** 3453 – 3456.

LINK, R.E., DESAI, K., HEIN, L., STEVENS, M.E., CHRUSCINSKI, A., BERNSTEIN, D., BARSH, G.S. & KOBILKA, B.K. (1996). Cardiovascular regulation in mice lacking α_2-adrenergic receptor subtypes b and c. *Science,* **273,** 803–805.

MACMILLAN, L.B., HEIN, L., SMITH, M.S., PIASCIK, M.T. & LIMBIRD, L.E. (1996). Central hypotensive effects of the α_{2a}-adrenergic receptor subtype. *Science,* **273,** 801–803.

MALMBERG, A.B. & BASBAUM, A.I. (1998). Partial sciatic nerve injury in the mouse as a model of neuropathic pain: behavioral and neuroanatomical correlates. *Pain,* **76,** 215–222.

MAXWELL, R.A., PLUMMEN, A.J., SCHNEIDER, F., POLAVSKI, H. & DAMICEL, A.I. (1960). Pharmacology of [2-(octahydro-1-azovinyl)-ethyl]-guanidine sulfate (guanethidine). *J. Pharmacol.,* **128,** 22–29.

MCLACHLAN, E.M., JÄNIG, W., DEVOR, M. & MICHAELIS, M. (1993). Nerve injury triggers noradrenergic sprouting within dorsal root ganglia. *Nature (Lond.),* **363,** 543–545.

MICHAELIS, M., DEVOR, M. & JÄNIG, W. (1996). Sympathetic modulation of activity in rat dorsal root ganglion neurons changes over time following peripheral nerve injury. *J. Neurophysiol.,* **76,** 753–763.

NEIL, A., ATTAL, N. & GUILBAUD, G. (1991). Effects of guanethidine on sensitization to natural stimuli and self-mutilating behaviour in rats with a peripheral neuropathy. *Brain Res.,* **565,** 237–246.

NICHOLAS, A.P., PIERIBONE, V. & HÖKFELT, T. (1993). Distributions of mRNAs for alpha-2 adrenergic receptor subtypes in rat brain: an in situ hybridization study. *J. Comp. Neurol.,* **328,** 575–594.

PANG, I.H. & VASKO, V.R. (1986). Morphine and norepinephrine, but not 5-hydroxytryptamine and gamma-aminobutyric acid inhibit the potassium-stimulated release of substance P from rat spinal cord slices. *Brain Res.,* **376,** 268–279.

PUKE, M.J., LUO, L. & XU, X.J. (1994). The spinal analgesic role of alpha 2-adrenoceptor subtypes in rats after peripheral nerve section. *Eur. J. Pharmacol.,* **260,** 227–232.

RAJA, S.N., TREEDE, R.D., DAVIS, K.D. & CAMPBELL, J.N. (1991). Systemic alpha-adrenergic blockade with phentolamine: a diagnostic test for sympathetically maintained pain. *Anesthesiology,* **74,** 691–698.

REDDY, S.V., MADERDRUT, J.L. & YAKSH, T.L. (1980). Spinal cord pharmacology of adrenergic agonist-mediated antinociception. *Pharmacol. Exp. Ther.,* **213,** 525–533.

RINGKAMP, M., GRETHEL, E.J., CHOI, Y., MEYER, R.A. & RAJA, S.N. (1999a). Mechanical hyperalgesia after spinal nerve ligation in rat is not reversed by intraplantar or systemic administration of adrenergic antagonists. *Pain,* **79,** 135–141.

RINGKAMP, M., ESCHENFELDER, S., GRETHEL, E.J., HABLER, H.J., MEYER, R.A., JANIG, W. & RAJA, S.N. (1999b). Lumbar sympathectomy failed to reverse mechanical allodynia- and hyperalgesia-like behavior in rats with L5 spinal nerve injury. *Pain,* **79,** 143–153.

ROSIN, D.L., TALLEY, E.M., LEE, A., STORNETTA, R.L., GAYLINN, B.D., GUYENET, P.G. & LYNCH, K.R. (1996). Distribution of alpha 2C-adrenergic receptor-like immunoreactivity in the rat central nervous system. *J. Comp. Neurol.,* **372,** 135–165.

ROSIN, D.L., ZENG, D., STORNETTA, R.L., NORTON, F.R., RILEY, T., OKUSA, M.D., GUYENET, P.G. & LYNCH, K.R. (1993). Immunohistochemical localization of alpha 2A-adrenergic receptors in catecholaminergic and other brainstem neurons in the rat. *Neuroscience,* **56,** 139–155.

SATO, J. & PERL, E.R. (1991). Adrenergic excitation of cutaneous pain receptors induced by peripheral nerve injury. *Science,* **251,** 1608–1610.

SELTZER, Z., DUBNER, R. & SHIR, Y. (1990). A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. *Pain,* **43,** 205–218.

SCADDING, J. (1981). Development of ongoing activity, mechanosensitivity and adrenaline sensitivity in severed peripheral axons. *Exp. Neurol.,* **73,** 345–364.

SHI, T.S., WINZER-SERHAN, U., LESLIE, F. & HOKFELT, T. (2000). Distribution and regulation of alpha(2)-adrenoceptors in rat dorsal root ganglia. *Pain,* **84,** 319–330.

SHIR, Y. & SELTZER, Z. (1991). Effects of sympathectomy in a model of causalgiform pain produced by partial sciatic nerve injury in rats. *Pain,* **45,** 309–320.

STONE, L.S., BROBERGER, C., VULCHANOVA, L., WILCOX, G.L., HOKFELT, T., RIEDL, M.S. & ELDE, R. (1998). Differential distribution of alpha2A and alpha2C adrenergic receptor immunoreactivity in the rat spinal cord. *J. Neurosci.,* **18,** 5928–5937.

STONE, L.S., MACMILLAN, L.B., KITTO, K.F., LIMBIRD, L.E. & WILCOX, G.L. (1997). The alpha2a adrenergic receptor subtype mediates spinal analgesia evoked by alpha2 agonists and is necessary for spinal adrenergic-opioid synergy. *J. Neurosci.,* **17,** 7157–7165.

STONE, L.S., VULCHANOVA, L., RIEDL, M.S., WANG, J., WILLIAMS, F.G., WILCOX, G.L. & ELDE, R. (1999). Effects of peripheral nerve injury on alpha-2A and alpha-2C adrenergic receptor immunoreactivity in the rat spinal cord. *Neuroscience,* **93,** 1399–1407.

SURPRENANT, A., HORSTMAN, D.A., AKBARALI, H. & LIMBIRD, L.E. (1992). A point mutation of the alpha 2-adrenoceptor that blocks coupling to potassium but not calcium currents. *Science,* **257,** 977–980.

TAKANO, M., TAKANO, Y. & YAKSH, T.L. (1993). 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,* **14,** 371–378.

TAKANO, Y. & YAKSH, T.L. (1992). Characterization of the pharmacology of intrathecally administered alpha-2 agonists and antagonists in rats. *J. Pharmacol. Exp. Ther.,* **261,** 764–772.

TAMSEN, A. & GORDH, T. (1984). Epidural clonidine produces analgesia. *Lancet,* **2,** 231–232.

TRACEY, D.J., CUNNINGHAM, J.E. & ROMM, M.A. (1995). Peripheral hyperalgesia in experimental neuropathy: mediation by a2-adrenoreceptors on post-ganglionic sympathetic terminals. *Pain,* **60,** 317–327.

WILLENBRING, S., BEAUPRIE, I.G. & DELEO, J.A. (1995). Sciatic cryoneurolysis in rats: a model of sympathetically independent pain, Part 1: Effects of sympathectomy. *Anesth. Analg.,* **81,** 544–548.

XIAO, X.H. & RAND, M.J. (1990). Effects of the alpha 2-adrenoceptor agonist UK14304 on pressor responses in pithed rats. *Clin. Exp. Pharmacol. Physiol.,* **17,** 725–734.

XIE, Y., ZHANG, J., PETERSEN, M. & LAMOTTE, R.H. (1995). Functional changes in dorsal root ganglion cells after chronic nerve constriction in the rat. *J. Neurophysiol.,* **73,** 1811–1820.

YAKSH, T.L. (1985). Pharmacology of spinal adrenergic systems which modulate spinal nociceptive processing. *Pharmacol. Biochem. Behav.,* **22,** 845–858.

YAKSH, T.L., POGREL, J.W., LEE, Y.W. & CHAPLAN, S.R. (1995). Reversal of nerve ligation-induced allodynia by spinal alpha-2 adrenoceptor agonists. *J. Pharmacol. Exp. Ther.,* **272,** 207–214.

(Received October 23, 2000
Revised February 12, 2001
Accepted February 13, 2001)