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Potentialiation of Spinal α_2 -Adrenoceptor Analgesia in Rats Deficient in TRPV1- Expressing Afferent Neurons

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Abstract

The α_2 -adrenoceptors (α_2 -ARs) are located on primary afferent terminals and on neurons in the spinal cord dorsal horn. However, their relative contribution to the analgesic effect of the α_2 -AR agonists is not known. In this study, we determined the role of certain presynaptic α_2 -ARs in the antinociceptive effect produced by intrathecal administration of the α_2 -AR agonist clonidine. TRPV1-expressing sensory neurons were removed by resiniferatoxin (RTX). The effect of intrathecal injection of clonidine was measured by testing the paw withdrawal response to noxious mechanical or heat stimuli. In RTX-treated rats, the α_{2A} -AR-immunoreactivity co-expressed with TRPV1-expressing terminals in the spinal cord was eliminated. However, the α_{2C} -AR-immunoreactivity in the spinal cord was little changed. Surprisingly, intrathecal administration of clonidine produced a much greater increase in the mechanical withdrawal threshold in RTX- than in vehicle-treated rats. The duration of the clonidine effect was also significantly increased in RTX-treated rats. Furthermore, in the vehicle-treated group, although intrathecal injection of clonidine produced a large increase in the thermal withdrawal latency, it only had a small and short-lasting effect on the mechanical withdrawal threshold. This study provides new information that the antinociceptive effect of spinally administered α_2 -AR agonists is largely modality-specific. Loss of TRPV1-expressing sensory neurons leads to a reduction in presynaptic α_{2A} -ARs but paradoxically potentiates the effect of clonidine on mechano-nociception.

INTRODUCTION

The spinal dorsal horn is a critical site for the transmission of nociceptive information. The α_2 -adrenoceptors (α_2 -ARs) in the spinal dorsal horn are involved in modulation of nociceptive information. For example, intrathecal administration of the α_2 -AR agonist clonidine produces analgesic effects in acute and chronic pain models (Pan et al., 1999; Yaksh et al., 1995). Also, epidural administration of clonidine is effective in the treatment of patients with chronic pain (Eisenach et al., 1995; Rauck et al., 1993). Three α_2 -ARs (α_{2A} -, α_{2B} -, and α_{2C} -ARs) have been cloned, and all of which are coupled to inhibitory G proteins (Bylund et al., 1992). Using mice deficient in α_{2A} -, α_{2B} -, or α_{2C} -ARs, it has been shown that the α_{2A} -ARs are primarily involved in the analgesic effect produced by α_2 -AR agonists (Fairbanks et al., 2002; Stone et al., 1997).

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Furthermore, the α_2 -AR in the spinal cord also contributes to the antinociceptive effect produced by intrathecal α_2 -AR agonists (Fairbanks et al., 2002).

The α_2 -ARs are located presynaptically on the central terminals of primary afferent neurons and postsynaptically on the spinal dorsal horn neurons (Stone et al., 1998). However, the relative role of these receptors in the analgesic effect of spinally administered α_2 -AR agonists is not fully known. The capsaicin receptor, transient receptor potential vanilloid type 1 (TRPV1), is critical in the detection of thermal nociception. Mice deficient in TRPV1 show a reduced nociceptive response to noxious heat (Caterina et al., 2000). Resiniferatoxin (RTX), originally extracted from the resin spurge *Euphorbia resinifera*, is an ultrapotent TRPV1 agonist. RTX removes TRPV1-expressing sensory neurons in adult rats and induces a long-lasting impairment of thermal nociception (Chen and Pan, 2006b; Pan et al., 2003). However, in the absence of TRPV1-expressing sensory neurons, mechano-nociception appears to be largely intact in RTX-treated rats (Chen and Pan, 2006b; Pan et al., 2003). Because pain elicited by noxious mechanical stimuli is more prevalent than thermal pain, it is important to study how mechanical nociceptive transmission through non-TRPV1 neurons is regulated at the spinal level in RTX-treated animals. Among the three types of α_2 -ARs, at least the α_{2A} -ARs are present on capsaicin-sensitive afferent terminals (Stone et al., 1998). Thus, removal of TRPV1-expressing afferent neurons by RTX could lead to a decrease in presynaptic α_{2A} -ARs in the spinal dorsal horn. But little is known about how reduction of presynaptic α_2 -ARs influences the antinociceptive effect produced by spinally administered α_2 -AR agonists.

In the present study, we determined the potential importance of presynaptic α_2 -ARs in the spinal analgesic effect of the α_2 -AR agonist clonidine. We found unexpectedly that although RTX eliminated presynaptic α_{2A} -ARs present on TRPV1-expressing afferent terminals, the effect of intrathecally injected clonidine on mechano-nociception was profoundly potentiated in RTX-treated rats. This study provides important new information about the distinct role of α_2 -ARs in the modulation of mechano-nociception and a possible heterogeneous mechanism involved in the signaling and desensitization of α_2 -ARs in the spinal cord.

METHODS

Animals

Male rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 200–220 g were used in this study. Rats received a single intraperitoneal injection of RTX (200 g/kg, LC Laboratories, Wolburn, MA) under isoflurane (2% in O₂) anesthesia. RTX was dissolved in a mixture of 10% Tween-80 and 10% ethanol in normal saline (Chen and Pan, 2006b; Pan et al., 2003). Rats in the control group received an intraperitoneal injection of the vehicle under isoflurane anesthesia. The experiments were conducted 4–5 weeks after the RTX and vehicle injections unless otherwise stated. The surgical preparations and experimental protocols were approved by the University of Texas M. D. Anderson Cancer Center and conformed to the National Institutes of Health guidelines on the ethical use of animals. All efforts were made to minimize both the suffering and number of animals used.

Intrathecal catheters (PE-10 polyethylene tubing) were inserted in RTX- and vehicle-treated rats during isoflurane-induced anesthesia. The catheters were advanced 8 cm caudal through an incision in the cisternal membrane and secured to the musculature at the incision site (Yaksh and Rudy, 1976). The rats were allowed to recover for 5–7 days before being used to test the antinociceptive effect of clonidine. Only animals with no evidence of neurological deficits after catheter insertion were studied. Drugs for intrathecal injection were dissolved in normal saline and administered in a volume of 5 μ l followed by a 10 μ l flush with normal saline. Repeated intrathecal injections in the same animals were separated by 4–5 days. Clonidine and yohimbine were obtained from Sigma (St. Louis, MO).

Behavioral assessment of thermal nociception

To quantitatively assess the thermal sensitivity, rats were placed on the glass surface of a thermal testing apparatus (IITC Life Sciences, Woodland Hills, CA). The rats were allowed to acclimate for 30 min before testing. The temperature of the glass surface was maintained at a constant 30°C. A mobile radiant heat source located under the glass was focused onto the hindpaw of each rat. The paw-withdrawal latency was recorded with a timer, and each hindpaw was tested once to obtain the average. A cut-off of 30 s was used to prevent potential tissue damage (Chen and Pan, 2003b,2006a).

Behavioral testing of mechanical nociception

The nociceptive mechanical threshold was measured using an Ugo Basile Analgesimeter (Varese, Italy). The test was performed by applying a noxious pressure to the hindpaw. By pressing a pedal that activated a motor, the force increased at a constant rate on the linear scale. When the animal displayed pain by withdrawal of the paw or vocalization, the pedal was immediately released, and the nociceptive threshold was read on a scale. A cut-off of 400 g was used to avoid potential tissue injury (Chen and Pan, 2003b,2006a). Both hindpaws were tested in each rat, and the mean value was used as the nociceptive withdrawal threshold.

Double immunofluorescence labeling of TRPV1 and α_{2A} - or α_{2C} -ARs

To determine the co-localization and relationship between TRPV1-positive neurons and α_2 -ARs in both RTX- and vehicle-treated rats, doubling labeling of TRPV1 and α_{2A} - or α_{2C} -ARs in the spinal dorsal horn was performed on three vehicle- and three RTX-treated rats 4 wk after treatment. Under deep anesthesia with intraperitoneal sodium pentobarbital (60 mg/kg), each rat was intracardially perfused with 250 ml of 4% paraformaldehyde in 0.1 M PBS (pH 7.4) and 200 ml of 10% sucrose in 0.1 M PBS (pH 7.4). The lumbar spinal cord were quickly removed and postfixed in the same fixative solution for 2 hr at room temperature and cryoprotected in 30% sucrose in PBS for 48 hr at 4°C. The tissues were cut to 25 μ m in thickness and collected free-floating in 0.1 M PBS. Sections intended for the labeling of TRPV1 and α_{2A} -AR were incubated in a mixture of primary antibodies (guinea pig anti-TRPV1, dilution: 1:1000; and rabbit anti- α_{2A} -AR, dilution 1:100, Neuromics, Minneapolis, MN) for 2 hr at room temperature and overnight at 4°C. The sections used for the double labeling of TRPV1 and α_{2C} -AR were incubated in another mixture of primary antibodies (rabbit anti-TRPV1, dilution 1:1000; and guinea pig anti- α_{2C} -AR, dilution 1:500, Neuromics). The specificity of the α_{2A} -AR and α_{2C} -AR primary antibodies was demonstrated previously (Stone et al., 1998). Subsequently, sections labeled with TRPV1 and α_{2A} -AR were incubated with a mixture of secondary antibodies (Biotin-SP conjugated to goat anti-rabbit IgG, dilution: 1:200, Jackson ImmunoResearch, West Grove, PA; and Alexa Fluor- 594 conjugated goat anti-guinea pig IgG, dilution 1:200, Molecular Probes, Eugene, OR). The remaining sections for TRPV1 and α_{2C} -AR labeling were incubated in a different mixture of secondary antibodies (Biotin-SP conjugated to goat anti-guinea pig IgG, dilution: 1:200, Jackson ImmunoResearch; and Alexa Fluor-594 conjugated to goat anti-rabbit IgG, dilution: 1:200, Molecular Probes) for 2 hr at room temperature. Following the secondary antibody incubation, all sections were rinsed and incubated with streptavidin-conjugated horseradish peroxidase (PerkinElmer, Boston, MA) for 1 hr at room temperature. Next, the sections were incubated with FITC-tyramide (PerkinElmer, Boston, MA) for 10 min at room temperature, and then mounted on slides, dried, and coverslipped. The sections were examined on a laser scanning confocal microscope (Carl Zeiss, Jena, Germany), and areas of interest were photodocumented.

To quantitatively evaluate the changes in α_2 -AR-immunoreactivity in the spinal cord by RTX, image analysis was performed on eight sections from each rat. A threshold intensity was determined for each image at which most of the background labeling was suppressed. The same region of the superficial dorsal horn (i.e., laminae I and II) was outlined in the sections from

vehicle- and RTX-treated rats (Chen and Pan, 2003a). The percentage of pixels within the area with an intensity above threshold was measured by a computer-based imaging analysis program (AIS; Imaging Research, Inc., St. Catharines, Ontario, Canada). The investigator performing the image analysis was blinded to the treatment group. Results are expressed as the percentage change in the fluorescence density in RTX-treated rats compared with that in vehicle-treated control rats.

Statistical analysis

Data were presented as mean $\pm$ S.E.M. Paw withdrawal thresholds in response to thermal stimulation before and after RTX or vehicle treatment were compared using a paired Student's *t* test. The effect of clonidine on the mechanical and thermal withdrawal threshold was determined using repeated measures ANOVA followed by Tukey's *post hoc* test. The difference in the maximal effect of clonidine between vehicle-treated and RTX-treated rats was compared using two-way ANOVA with Bonferroni's *post hoc* test. To calculate ED₅₀, data were converted to the percentage of the effect of clonidine based on the following calculation: $[(\text{maximal effect} - \text{baseline})/(\text{cutoff} - \text{baseline})] \times 100\%$. The ED₅₀ values of clonidine and the 95% confidence limits were determined by nonlinear regression analyses of the dose-response using GraphPad Prism (GraphPad Software, San Diego, CA). $P < 0.05$ was considered statistically significant.

RESULTS

Systemic injection of RTX caused a large increase in the paw-withdrawal latency in response to the noxious heat stimulus. One week after RTX treatment, the thermal paw withdrawal latency was significantly increased from 9.36 ± 1.24 to 25.93 ± 2.31 s ($n = 21$). In vehicle-treated rats, however, there was no significant difference in the sensitivity (from 11.06 ± 1.34 to 9.56 ± 1.47 s, $n = 20$).

Effect of RTX on α_{2A} -AR-immunoreactivity in the spinal dorsal horn

To examine the effect of RTX treatment on the α_{2A} -AR-immunoreactivity in the spinal cord, double fluorescent labeling of TRPV1 and α_{2A} -AR was conducted in the lumbar spinal cord sections 4 wk after RTX or vehicle treatment. In vehicle-treated rats, the TRPV1-immunoreactivity was present in the superficial dorsal horn of the spinal cord, with a dense labeling in the lamina I and the outer zone of lamina II (Fig. 1). Also, the α_{2A} -AR-immunoreactivity was abundant in the spinal superficial dorsal horn. The co-localization of TRPV1- and α_{2A} -AR-immunoreactivities was evident in both lamina I and the outer zone of lamina II, as shown in both low- and high-magnification confocal images (Fig. 1). In RTX-treated rats, the TRPV1-immunoreactivity was completely absent in the superficial lamina. The reduction in α_{2A} -AR-immunoreactivity was clearly visible, especially in spinal laminae I and II in RTX-treated rats (Fig. 1). Semi-quantitative imaging analysis showed a $64.7 \pm 8.4\%$ reduction in α_{2A} -AR-immunoreactivity in spinal laminae I and II in RTX-treated rats compared with that in vehicle-treated rats.

Effect of RTX on α_{2C} -AR-immunoreactivity in the spinal dorsal horn

To assess the effect of RTX treatment on the α_{2C} -AR-immunoreactivity in the spinal cord, double fluorescent labeling of TRPV1 and α_{2C} -AR was performed in the lumbar spinal cord sections 4 wk after treatment. The α_{2C} -AR-immunoreactivity was present throughout the spinal cord dorsal horn in vehicle-treated rats (Fig. 2). When visualized from low-magnification confocal images, there appeared to be some co-localization of TRPV1- and α_{2C} -AR-immunoreactivities in the lamina I. However, when high-magnification confocal images were examined, most α_{2C} -AR-immunoreactive punctate in the superficial dorsal horn appeared to be located on neurons and was not co-localized with TRPV1-immunoreactive terminals (Fig.

2). In RTX-treated rats, there was no evident reduction in α_2C -AR-immunoreactivity in the spinal dorsal horn although the TRPV1-immunoreactivity was absent (Fig. 2). Compared with that in vehicle-treated rats, there was no apparent change ($3.2 \pm 4.9\%$) in α_2C -AR-immunoreactivity in the superficial dorsal horn of RTX-treated animals.

Effect of intrathecal clonidine on mechanical nociception in RTX- and vehicle-treated rats

Because RTX treatment removed the α_{2A} -AR-immunoreactivity co-localized with TRPV1-expressing afferent terminals in the spinal cord, we initially tested the effect of clonidine on the mechanical withdrawal threshold in 8 RTX-treated and 7 vehicle-treated rats 4 wk following their treatment. The baseline threshold in RTX-treated rats was significantly lower than that in the vehicle group (132.3 ± 6.1 vs. 155.3 ± 5.4 g, $P < 0.05$). Intrathecal administration of 5–20 g of clonidine increased the paw withdrawal threshold, as measured with a noxious pressure stimulus, in a dose-dependent manner in both groups (Fig. 3). However, in vehicle-treated rats, only 20 g of clonidine produced a significant effect. Intrathecal injection of 5–20 g of clonidine produced a significantly greater effect on the paw withdrawal threshold in RTX- than in vehicle-treated rats (Fig. 3). Intrathecal pre-treatment with 30 g of yohimbine, a specific α_2 -AR antagonist, completely blocked the effect of 20 g of clonidine in RTX-treated rats. In the control group, the peak clonidine effect at different doses appeared within 15 min, and the effect of 20 g of clonidine was rapidly disappeared within 45 min after intrathecal injection (Fig. 3A). By comparison, in the RTX group, the maximal effect of clonidine in all doses was observed at 30 min after injection, and the effect of 20 g of clonidine lasted more than 90 min (Fig. 3B). The estimated ED_{50} (95% confidence limits) of the effect of intrathecal clonidine for the RTX and vehicle groups were 7.13 (2.39–11.83) and 18.27 (12.64–45.62) g, respectively.

Effect of intrathecal clonidine on thermal nociception in vehicle-treated rats

Because intrathecal clonidine produced a small and short-lasting effect on the mechanical withdrawal threshold in vehicle-treated rats, we also examined the effect of intrathecal clonidine on the paw withdrawal latency in response to a noxious heat stimulus in another 8 vehicle-treated rats. Intrathecal administration of 5, 10, and 20 g of clonidine significantly increased the paw withdrawal latency in a dose dependent manner (Fig. 4). The ED_{50} (95% confidence limits) of the effect of intrathecal clonidine on the withdrawal latency were 8.56 (4.91–17.66) g. Notably, the maximal clonidine effect and its duration on the thermal withdrawal latency were much greater than those found for the mechanical threshold test (Figs. 3A and 4).

DISCUSSION

This is the first study that determined the role of presynaptic α_2 -ARs in the spinal cord in the analgesic effect produced by clonidine. We found that RTX eliminated presynaptic α_{2A} -ARs that are co-localized with TRPV1-expressing afferent terminals in the spinal dorsal horn. Unexpectedly, the antinociceptive effect of clonidine on mechano-nociception was largely potentiated in RTX-treated rats. Furthermore, we observed that intrathecal clonidine produced a much greater effect on thermal nociception than on mechanical nociception in vehicle-treated rats. Therefore, these findings provide new information for our understanding of the role of subpopulations of α_2 -ARs in the regulation of modality-specific nociceptive information in the spinal cord. These data also suggest a possible heterogeneous mechanism involved in the signaling and desensitization of α_2 -ARs in TRPV1- and non-TRPV1-expressing sensory neurons.

We found that intrathecal injection of clonidine produced a large increase in the thermal nociceptive threshold. By comparison, the effect of intrathecal clonidine on the mechano-

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nociceptive threshold was small and short-lasting. Similar to this finding, intrathecal administration of muscarinic receptor agonists also has a more pronounced effect on thermal nociception than on mechanical nociception in rats (Chen and Pan, 2003b). The modality-specific nociception is likely due to the fact that many receptors and ion channels are differentially distributed in different populations of sensory neurons in the dorsal root ganglia (Bennett et al., 1998; Caterina et al., 2000; Snider and McMahon, 1998; Vydyanathan et al., 2005; Wu and Pan, 2004a,b). TRPV1-expressing primary afferent neurons are critically involved in detection of noxious heat (Chen and Pan, 2006b; Pan et al., 2003). Because many α_{2A} -ARs in the spinal cord are present on TRPV1-expressing afferent terminals, this could explain why intrathecal clonidine is more effective on thermal nociception than on mechanical nociception. Because of the diminished thermal sensitivity in RTX-treated rats, we did not test the effect of clonidine on thermal nociception. We have shown previously that activation of α_2 -ARs with clonidine can reduce glutamate release from primary afferent terminals (Pan et al., 2002), which likely accounts for the analgesic effect of α_2 -AR agonists in the spinal cord. This inhibitory effect of α_2 -AR agonists on spinal synaptic transmission could be due to inhibition of voltage-gated calcium channels through pertussis toxin-sensitive G proteins (Nah et al., 1993). Therefore, the distinct antinociceptive effect of clonidine on thermal nociception is probably mediated primarily by presynaptic α_{2A} -ARs in the spinal cord.

The most salient finding of our study is that the effect of intrathecal clonidine on mechanonociception was potentiated despite the fact that the presynaptic α_{2A} -ARs were reduced in RTX-treated rats. The effect of RTX seems limited to dorsal root ganglion neurons and primary afferent terminals in the spinal dorsal horn because the dorsal horn neurons expressing TRPV1 are not significantly affected (Chen and Pan, 2006b). In this study, we observed that RTX had a different effect on the level of α_{2A} -ARs and α_{2C} -ARs in the spinal cord. In the spinal cord dorsal horn, there was an extensive co-localization of α_{2A} -ARs with TRPV1-expressing afferent terminals. In contrast, little co-localization of TRPV1 and α_{2C} -ARs was observed in the spinal cord. As a result of degeneration of TRPV1-expressing afferent terminals in RTX-treated rats, the level of α_{2A} -, but not α_{2C} -, ARs was largely reduced in the spinal dorsal horn. The localization of the α_{2A} -, but not the α_{2C} -, ARs on capsaicin-sensitive afferent terminals has been shown in the rat spinal cord using neonatal capsaicin treatment (Stone et al., 1998). It should be noted that the use of RTX in adult rats is a preferred approach since it partially avoids developmental changes after capsaicin injection in neonatal animals. Because there are no specific antibodies for the α_{2B} -AR subtype, we were unable to examine the distribution of α_{2B} -ARs in the spinal cord. Although the α_{2B} -AR mRNA has been detected in some neurons in the rat spinal dorsal horn (Shi et al., 1999), this subtype appears not involved in the analgesic effect of α_2 -AR agonists (Fairbanks et al., 2002). Radioligand binding studies suggest that the α_{2A} -AR is the predominant subtype in the spinal cord of rats and humans (Lawhead et al., 1992; Uhlen and Wikberg, 1991). Thus, considering the substantial decrease in α_{2A} -ARs in the spinal dorsal horn, the potentiation of the analgesic effect of intrathecal clonidine on mechanonociception was not anticipated in RTX-treated rats. Since this effect was completely blocked by intrathecal administration of yohimbine, a specific α_2 -AR antagonist, the effect of clonidine on mechanonociception is mediated by spinal α_2 -ARs. However, due to lack of highly selective agonists and antagonists for each α_2 -AR subtype, the relative contribution of the individual α_2 -AR subtypes to the analgesic effect produced by spinally administered α_2 -AR agonists is currently unclear. Data from this study suggest that mechanonociception, transduced through non-TRPV1 sensory neurons, is subject to potent modulation by α_2 -ARs in the spinal cord following removal of TRPV1-expressing primary afferents.

Consistent with our recent finding on the paradoxical effect of RTX on spinal opioid receptors and opioid analgesia (Chen and Pan, 2006b), this current study also shows that the degree of the effect of intrathecal clonidine on mechanonociception is not proportional to the number of α_{2A} -ARs in the spinal dorsal horn. The mechanisms underlying this potentiated analgesic

potency of intrathecal clonidine in RTX-treated rats are not well understood. Both α_{2A} - and α_{2C} -ARs in the spinal cord are involved in the analgesic effect of α_2 -AR agonists (Fairbanks et al., 2002; Stone et al., 1997). It is possible that when the α_{2A} -ARs present on TRPV1-expressing afferent terminals are removed by RTX, the effect of clonidine on mechanical nociception may be mediated mainly through the remaining α_{2A} -ARs and/or α_{2C} -ARs in the spinal cord. Another possibility is that the nociceptive input is reduced due to loss of TRPV1-expressing afferent neurons in RTX-treated rats, and this reduced sensory inflow can be inhibited more effectively by the remaining α_2 -ARs in the spinal cord. Alternatively, more high-affinity α_2 -ARs may be present on mechano-nociceptive/non-TRPV1- than TRPV1-expressing afferent terminals in the spinal cord. However, this seems less likely because clonidine only had a minimal effect on mechano-nociception in vehicle-treated rats. An interesting similarity is that spinally administered clonidine has a profound effect on allodynia in animal models of neuropathic pain (Pan et al., 1999; Yaksh et al., 1995), although nerve ligation injury decreases the α_{2A} -ARs in the spinal cord (Stone et al., 1999). This potent antiallodynic effect of clonidine may be due to increased coupling of α_2 -ARs to G proteins or their interaction with muscarinic acetylcholine receptors in the spinal cord after nerve injury (Bantel et al., 2005; Pan et al., 1999).

The possibility that different signaling mechanisms are involved in the desensitization of α_2 -ARs in TRPV1- and non-TRPV1-expressing sensory neurons should also be acknowledged. The remaining α_2 -ARs in the spinal cord may become more resistant to desensitization by α_2 -AR agonists in RTX-treated rats. Notably, the increased duration of the clonidine effect and the delayed peak effect (from 15 to 30 min) in RTX-treated rats suggest that the potentiated clonidine effect could be due to reduced desensitization of α_2 -ARs in the spinal cord. The α_2 -ARs can be desensitized after activation by α_2 -AR agonists through phosphorylation by G-protein-coupled receptor kinases (Jewell-Motz and Liggett, 1996; Pao and Benovic, 2005). Arrestins can bind to phosphorylated α_2 -ARs and cause internalization of α_2 -ARs (DeGraff et al., 1999). Protein kinase C (PKC) is also capable of phosphorylating α_2 -ARs (Liang et al., 1998; Liang et al., 2002). Activation of PKC attenuates the inhibitory effect of clonidine on voltage-gated calcium channels (Attali et al., 1991). It has been shown that stimulation of PKC can rapidly desensitize α_2 -ARs to attenuate the effect of α_2 -AR agonists (Convents et al., 1989; Jansson et al., 1994). Because of the possible differential expression of PKC in TRPV1- and non-TRPV1-expressing afferent neurons/terminals, this may substantially influence the efficacy of α_2 -AR agonists. Importantly, mice deficient in PKC γ are resistant to development of analgesic tolerance by chronic administration of either morphine or clonidine (Zeitl et al., 2001). We have shown that the PKC γ -immunoreactive terminals in the superficial dorsal horn were substantially reduced in RTX-treated rats. Thus, reduced desensitization of α_2 -ARs through PKC in the spinal cord may account, at least in part, for the increased potency and duration of the effect of α_2 -AR agonists on mechano-nociception (Chen et al., 2007). The pre- and postsynaptic α_2 -ARs in the spinal cord may be subject to heterogeneous desensitization by PKC. Further studies are required to determine whether desensitization of α_2 -ARs in TRPV1- and non-TRPV1-sensory neurons is differentially regulated by PKC. The findings of this study are important for our understanding of the role of pre- and postsynaptic α_2 -ARs in the spinal cord in regulation of modality-specific nociception.

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List of abbreviations

DRG

dorsal root ganglion

α₂-Ars

	α_2 -adrenoceptors
PKC	protein kinase C
IB₄	<i>Griffonia simplicifolia</i> isolectin B ₄
RTX	resiniferatoxin
TRPV1	transient receptor potential vanilloid type 1

Fig 1. Confocal images showing the effect of RTX on α_{2A} -AR- and TRPV1-immunoreactivity in the spinal cord dorsal horn. Representative confocal images showing α_{2A} -AR- and TRPV1-immunoreactivities in the spinal dorsal horn of one vehicle- and one RTX-treated rat. All images are single confocal optical sections. Scale bar, 100 μ m. Inset: high-magnification images (scale bar = 10 μ m) showing co-localization of α_{2A} -AR- and TRPV1-immunoreactivity in the lamina I and II. Co-localization is indicated in yellow when two images are digitally merged.

Fig 2.

Double immunofluorescence labeling showing α_2C -AR- and TRPV1-immunoreactivity in the spinal cord dorsal horn of one vehicle-treated and one RTX-treated rat. Representative confocal images showing α_2C -AR- and TRPV1-immunoreactivities in the spinal dorsal horn of one vehicle- and one RTX-treated rat. All images are single confocal optical sections. Scale bar, 100 μ m. Inset: high-magnification images (scale bar = 10 μ m) showing the distribution of α_2C -AR- and TRPV1-immunoreactivity in the lamina I and II.

Fig 3.

Effect of intrathecal clonidine on the mechano-nociceptive threshold in RTX- and vehicle-treated rats. A, time course of the effect of intrathecal injection of 5, 10, and 20 g of clonidine in 7 vehicle-treated rats. B, time course of the effect of intrathecal injection of 5, 10, and 20 g of clonidine in 8 RTX-treated rats. Note that intrathecal injection of 20 g of clonidine failed to alter significantly the withdrawal threshold in the presence of 30 g of yohimbine. The nociceptive threshold was determined by the withdrawal response of the hindpaw to a noxious pressure stimulus. Data are represented as means $\pm$ S.E.M. *, $P < 0.05$ compared with the pretreatment control.

Fig 4.

Time course of the effect of intrathecal injection of 5, 10, and 20 g of clonidine on thermal nociception in vehicle-treated rats ($n = 8$). The nociceptive threshold was determined by the withdrawal latency of the hindpaw to a noxious heat stimulus. Data are shown as means $\pm$ S.E.M. *, $P < 0.05$ compared with the pretreatment control.