Anti-allodynic effects following intrathecal administration of α_1- and α_2-adrenergic receptor agonists in a rat model of trigeminal neuropathic pain

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Abstract

The descending noradrenergic system inhibits nociception in the spinal cord. Spinal nerve injury triggers ectopic sprouting of sympathetic nerve fibers within dorsal root ganglia. Spinal α_2-adrenoceptors, but not α_1-adrenoceptors, exert an inhibitory effect in a rat model of spinal nerve ligation. However, trigeminal nerve injury does not induce sprouting of sympathetic nerve fibers in the trigeminal ganglion. In the present study, we analyzed the roles of α_1- and α_2-adrenoceptors in anti-allodynic effects following trigeminal nerve injury.

Chronic constriction injury to the infraorbital nerve (ION-CCI) with loose ligatures was used to establish a trigeminal neuropathic pain model. Allodynia was evaluated by applying von Frey filaments. The anti-allodynic effects after intrathecal administration of α_1- and α_2-adrenoceptor agonists and antagonists were examined.

Administration of the α_1-adrenoceptor agonist phenylephrine (3, 10, and 30 μg) and α_2-adrenoceptor agonist clonidine (3, 10, and 30 μg) resulted in dose-dependent anti-allodynic effects. Intrathecal administration of the α_1-adrenoceptor antagonist prazosin (30 μg) and α_2-adrenoceptor antagonist yohimbine (30 μg) did not alter the mechanical thresholds. Intrathecal pretreatment with prazosin (3 and 10 μg) reduced the anti-allodynic effects of the highest phenylephrine dose, while intrathecal pretreatment with yohimbine (3 and 10 μg) reduced the anti-allodynic effects of the highest clonidine dose.

Peripheral adrenergic modulation following nerve injury did not aggravate trigeminal neuropathic pain. These results differ from a previous rat neuropathic pain model of spinal nerve injury. In the

ION-CCI rat model, the spinal α_1- and α_2-adrenoceptors played roles in spinal inhibition of trigeminal neuropathic pain.

Keywords: infraorbital nerve, trigeminal nerve, α_1- and α_2-adrenergic receptor, orofacial neuropathic pain, nerve injury, sympathetic sprouting

Abbreviations: Chronic constriction injury (CCI), Infraorbital nerve (ION), Dimethylsulfoxide (DMSO), Area under time-course curves (AUC), Nucleus raphe magnus (NRM), Lateral reticular nucleus (LRN), Locus coeruleus (LC)

1. Introduction

Transmission of nociceptive information in the spinal cord is subject to modulation by many different neural systems. The descending noradrenergic system inhibits nociception in the spinal cord (Petrovaara, 2006; Yoshimura and Furue, 2006), and noradrenaline application to the spinal cord results in an anti-nociceptive effect (Howe et al., 1983; Reddy et al., 1980). In addition, intrathecal administration of α_2-adrenoceptor agonists produces anti-nociception in experimental animal models (Asano et al., 2000; Fisher et al., 1991; Post et al., 1987; Reddy et al., 1980; Solomon et al., 1989; Takano and Yaksh, 1992). In the spinal cord, noradrenaline is released from descending pathways, which suppresses pain *via* α_2-adrenergic activation on central terminals of primary afferent nociceptors (pre-synaptic inhibition), as well as direct α_2-adrenergic action on pain-relay neurons (post-synaptic inhibition) (Petrovaara, 2006; Yoshimura and Furue, 2006). Studies have shown that pharmacological activation of α_1-adrenoceptors induces behavioral anti-nociception (Aran et al.,1990; Howe et al., 1983; Orii et al., 2002; Reddy et al., 1980; Tasker et al., 1992), and noradrenaline release in the spinal cord increases inhibition of synaptic transmission in the spinal dorsal horn *via* α_1-adrenoceptor-mediated activation of GABAergic and glycinergic inhibitory interneurons (Baba et al., 2002a, 2002b; Gassner et al., 2009; Yuan et al., 2009).

Peripheral nerve injury induces a neuropathic condition that involves allodynia, hyperalgesia, and spontaneous pain (Gracely et al., 1992; MacFarlane et a., 1997) as a result of injury-induced plastic changes in endogenous pain-modulating systems. These symptoms, which are common in patients with neuropathic pain, are often poorly relieved by conventional treatments, such as non-steroidal anti-inflammatory drugs and opioids (Arner and Meyerson, 1988; MacFarlane et al., 1997; Orii et al., 2002). In healthy peripheral tissues, noradrenaline has little effect on pain (Ali et al., 2000; Davis et al., 1991; Fuchs et al., 2001; Torebjork et al., 1995). However, nerve injury induces sympathetic sprouting in dorsal root ganglia (Chung et al., 1993;

McLachlan et al., 1993; Ramer and Bisby, 1997). In injured peripheral tissue, noradrenaline aggravates pain (Davis et al., 1991; Torebjork et al.,1995), which might contribute to development of chronic neuropathic pain. Intrathecal injection of an α_2-adrenoceptor agonist, but not an α_1-adrenoceptor agonist, attenuates mechanical hypersensitivity in a rat model of spinal nerve ligation (Yaksh et al., 1995). In addition, peripheral nerve injury decreases spinal anti-nociceptive efficacy of α_1-adrenergic compounds.

Trigeminal nerve injury does not induce ectopic sprouting of sympathetic fibers in the trigeminal ganglion (Benoliel et al., 2001), and the incidence of sympathetically evoked pain syndromes is significantly less following injuries to the face or jaws, compared with limb injuries (Matthews, 1989). Noradrenaline mediates descending inhibition of nociceptive responses in neurons of the rat spinal trigeminal nucleus (Cahusac et al., 1995), and intrathecal administration of α_2-adrenoceptor agonists has been shown to inhibit trigeminal nociception (Wang et al., 2002; Zhang et al., 1998). In the spinal trigeminal nucleus, noradrenaline released from descending pathways suppresses pain *via* inhibitory action on α_2-adrenoceptors (Grudt et al., 1995). Noradrenaline also increases inhibition of synaptic transmission in the substantia gelatinosa *via* α_1-adrenoceptor-mediated activation of GABAergic and glycinergic inhibitory interneurons (Grudt et al., 1995). However, the anti-allodynic effect of adrenergic receptors in a model of trigeminal neuropathic pain remains unclear.

Chronic constriction injury to the infraorbital nerve (ION-CCI) with loose ligatures has been commonly used to reliably reproduce a model of trigeminal neuropathic pain (Benoliel et al., 2001; Idanpaan-Heikkila and Guilbaud, 1999; Latremoliere et al., 2008; Nakai et al., 2010a, 2010b, Nakae et al., 2008; Vos et al., 1994). Using an ION-CCI rat model, the present study analyzed the potential anti-allodynic effects from intrathecal administration of the α_1-adrenoceptor agonist phenylephrine and the α_2-adrenoceptor agonist clonidine. In addition, the role of α_1- or α_2-adrenoceptor in these effects was assessed by determine whether the anti-allodynic effects were prevented by intrathecal administration of the α_1-adrenoceptor antagonist prazosin or the α_2-adrenoceptor antagonis yohimbine.

2. Methods

2.1. Animals

All surgical and experimental protocols in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Osaka Medical College and were performed according to the National Institutes of Health guidelines. The study also conformed to the Guidelines of the International Association for the Study of Pain (Zimmermann, 1983). In particular, the experimental duration was kept as short as possible, and as few animals as possible were used. Male Sprague Dawley (SD) rats (body weight at time of surgery 180–220 g) were allowed free access to chow and tap water and were housed at 22 ± 2°C in a 12-h light–dark cycle (lights on from 08:00 to

20:00). Prior to surgery, the rats were allowed at least one week to habituate to the housing facilities.

2.2. Surgical procedures

According to a previously described method (Vos et al., 1994), the injured rats underwent unilateral chronic constriction injury to the right ION performed under a surgical microscope. In brief, the animals were anesthetized with sodium pentobarbital (Nembutal, 60 mg/kg i.p.), and a midline scalp incision was made to expose the skull and nasal bone. The edge of the orbit, which is formed by maxillary, frontal, lacrimal, and zygomatic bones, was separated, and the ION was separated at the most rostral extent in the orbital cavity, just caudal to the infraorbital foramen. Two nylon (5-0) ligatures (2 mm apart) were loosely tied around the ION. To obtain the desired degree of constriction, a criterion formulated by Bennet and Xie (Bennett and Xie, 1988) was applied: the ligature was used to reduce the nerve diameter by a small amount to decrease, but not interrupt, epineural circulation *via* the superficial vasculature. The scalp incision was closed in layers using nylon sutures (5-0). Following surgery, the rats were housed with free access to food and water and were allowed to recover for at least seven days. Tactile allodynia of the ligated nerve territory was confirmed by measuring the mechanical response threshold to von Frey filaments. Only rats with hyper-responsiveness to mechanical stimulation were utilized for the study. Subsequently, an intrathecal catheter was implanted for upper cervical spinal injection of drugs following sodium pentobarbital anesthesia (Nembutal, 60 mg/kg i.p.) (Ahn et al., 1998; Yaksh and Rudy, 1976). A polyethylene tube (PE10) was advanced 10 mm caudally through a small hole in the atlanto-occipital membrane and dura (Nakai et al., 2010a). The catheter was then surgically anchored to the surrounding musculature to maintain position. Rats with evidence of neuromuscular dysfunctions were immediately sacrificed, and the remaining rats were allowed to recover for seven days prior to drug testing.

2.3. Behavioral analysis

All behavioral assessments were performed in a quiet room, generally between 09:00 and 16:00. A series of von Frey filaments were used to determine pain hypersensitivity to mechanical stimulation. To observe behavioral responses to mechanical stimulation, the rats were individually placed in a plastic cage (25 × 40 × 18 cm) with bedding. After the rats were acclimated for 1 h, von Frey filaments (bending force of 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 15.0 g) were applied to skin innervated by the injured ION near the center of vibrissal pad and surrounding the mystacial vibrissae. Each von Frey filament was applied five times to the same region at approximately 1-s intervals. Head withdrawals, and touching or scratching of facial regions, were quantified as positive pain responses(Piao ZG et al., 2006). The response threshold was defined as the lowest filament force to produce at least

three positive responses in five trials. If no pain response was elicited with the 15-g filament, the threshold was recorded as 15-g. Motor dysfunction was evaluated using the righting reflex, stepping reflex, tail movement, posture, and ambulation. Sedation was determined by spontaneous activity, such as walking, standing, and grooming.

2.4. Experimental protocols and drugs

The first series of experiments examined the time course of anti-allodynic effects and dose-response effects of intrathecally administered α_1- and α_2-adrenoceptor agonists. The α_1-adrenoceptor agonist was (R)-(-)-phenylephrine hydrochloride (3, 10, and 30 μg), and the α_2-adrenoceptor agonist was clonidine hydrochloride (3, 10, and 30 μg). The second series of experiments evaluated time course of mechanical threshold following intrathecal administration of α_1- and α_2-adrenoceptor antagonists. The α_1-adrenoceptor antagonist was prazosin hydrochloride (30 μg), and the α_2-adrenoceptor antagonist was yohimbine hydrochloride (30 μg). The third series of experiments determined the effects of intrathecal pretreatment with prazosin (3 or 10 μg) on anti-allodynic action of phenylephrine, as well as the effects of intrathecal pretreatment with yohimbine (3 or 10 μg) on anti-allodynic clonidine action. All drugs were purchased from Wako Pure Chemical Industries (Osaka, Japan). Phenylephrine and clonidine were dissolved in saline, and prazosin and yohimbine were dissolved in 50% dimethylsulfoxide (DMSO). The drugs were delivered in 10-μl solution followed by 10-μl saline to flush the catheter. After determining baseline values, withdrawal thresholds were measured at 30, 60, 90, and 120 min after drug injection. In antagonist studies, antagonist was administered first and agonist was injected 10 min later.

2.5. Data analysis and statistics

Data were expressed as mean ± S.E.M. Time-course data are presented as withdrawal threshold. Percentage of maximum possible effect (% MPE) was calculated using the following formula, % MPE = (post-drug threshold − pre-drug threshold)/(15g − pre-drug threshold) × 100. The areas under time-course curves (AUC) for % MPE were calculated from individual scores at each time point, divided by the maximum score that was obtained over a 120-min observation period (% maximum possible AUC). The respective ED50 values and 95% confidence intervals (CI) were calculated using linear regression. Time course data for dose-response effects of the agonists were analyzed using two-way analysis of variance, and statistical differences were calculated using the Tukey-Kramer multiple-comparison test. For antagonist studies, the % maximum possible AUC of agonists, as well as % maximum AUC observed in the presence of the antagonist, were compared using an analysis of variance, and statistical differences were calculated using the Tukey-Kramer multiple-comparison test. Statistical

significance was set to $P < 0.05$.

3. Results

3.1. Anti-allodynic effects of α_1-adrenoceptor agonists

Intrathecal administration of the α_1 adrenoceptor agonist phenylephrine resulted in dose-dependent anti-allodynic effects. Figure 1A shows the time course of anti-allodynic effects. From 30 to 120 min after treatment with 30 µg phenylephrine, mechanical thresholds were significantly greater than with 3 or 10 µg agonist. Following treatment with 10 µg phenylephrine, mechanical thresholds weresignificantly greater at 30-90 min, compared with treatment with 3 µg agonist. Peak effects of intrathecal administration occurred at 30 min after injection. Motor function was assessed using the righting reflex, stepping reflex, tail movement, posture, and ambulation, which were normally preserved following intrathecal injection of all phenylephrine doses. In addition, sedation was not observed following treatment with phenylephrine.

3.2. Mechanical thresholds following α_1-adrenoceptor antagonist treatment

Intrathecal administration of 30 µg prazosin, an α_1-adrenoceptor antagonist, did not alter the mechanical threshold or exhibit any intrinsic effects. Intrathecally administered vehicle (50% DMSO) also had no effect on mechanical threshold. Figure 1B shows the time course for mechanical threshold of prazosin and 50% DMSO Prevention against anti-allodynic action of α_1-adrenoceptor agonist *via* an α_1-adrenoceptor antagonist Intrathecal pretreatment with prazosin affected phenylephrine anti-allodynic action; the maximum phenylephrine dose significantly reduced the anti-allodynic effect of 3 and 10 µg of prazosin (Fig. 1C).

3.3. Anti-allodynic effects of α_2-adrenoceptor agonists

Intrathecal administration of the α_2-adrenoceptor agonist clonidine resulted in anti-allodynic effects in a dose-dependent manner. Figure 2A shows the time course of anti-allodynic effects of the agonist. From 30 to 120 min after treatment with 30 µg of clonidine, mechanical thresholds were significantly greater than with 3 or 10 µg of agonist. After 10-µg clonidine treatment, mechanical thresholds were significantly greater from 30 to 60 min, compared with treatment with 3 µg of agonist. The peak effects of clonidine occurred 30 min after injection, and no motor dysfunction was observed. In the 30-µg clonidine group, sedation was noted from 30-60 min in three of eight rats; the rats were quiet and did not respond to the environment, spontaneous activity was decreased, and urinary avoidance was observed.

3.4. Mechanical thresholds of the α_2-adrenoceptor antagonist

Intrathecal administration of 30 µg of yohimbine, an α_2-adrenoceptor antagonist, did not alter mechanical threshold and did

not exhibit any intrinsic effects. In addition, intrathecal administration of vehicle (50% DMSO) exhibited no effect on mechanical threshold. Figure 2B shows the time course for mechanical threshold of yohimbine and 50% DMSO

3.5. Prevention against anti-allodynic action of α_2-adrenoceptor agonist *via* an α_2-adrenoceptor antagonist

Intrathecal pretreatment with yohimbine affected anti-allodynic action of clonidine (Fig. 2C). The anti-allodynic effect of the maximum clonidine dose was significantly reduced by 3 or 10 μg of yohimbine.

3.6. Anti-allodynic effects of α_1- and α_2-adrenoceptor agonists

Figure 3 shows the dose-response relationship between peak effect of the α_1-adrenoceptor agonist phenylephrine and the α_2-adrenoceptor agonist clonidine. The anti-allodynic ED50 (95% CI) values of phenylephrine and clonidine were 9.31 μg (5.62−13.00 μg) and 10.17 μg (6.42−13.92 μg), respectively. The ED50 values of phenylephrine and clonidine were similar.

4. Discussion

The present study analyzed anti-allodynic effects in a rat model of ION-CCI, together with intrathecal administration of α_1- (phenylephrine) and α_2-adrenoceptor (clonidine) agonists. Intrathecal pretreatment with the α_1-adrenoceptor antagonist prazosin attenuated the effects of phenylephrine, and intrathecal pretreatment with the α_2-adrenoceptor antagonist yohimbine attenuated the effects of clonidine. Prazosin binds to α_{2B}- or α_{2C}-adrenoceptors (Bylund et al., 1988), and clonidine exhibits affinity for the α_1-adrenoceptor receptor (Hayes et al., 1986; Tasker et al., 1992). However, the effects of α_1- and α_2-adrenoceptor agonists were significantly reduced following exposure to α_1- or α_2-adrenoceptor antagonists, respectively. These results suggested that spinal α_1- and α_2-adrenoceptor play a role in spinal inhibition of trigeminal neuropathic pain.

Studies have shown that the spinal α_2-adrenoceptor, but not spinal α_1-adrenoceptor, results in anti-allodynic effects in a rat model of spinal nerve ligation (Pan et al., 1999; Xu et al., 2000; Yaksh et al., 1995). Spinal nerve injury reduces spinal anti-nociceptive efficacy of α_1-adrenergic compounds. Adrenergic spinal pain modulation is different between trigeminal and spinal nerve injuries, and this difference is presumed to be caused by two factors: 1) injury-induced plastic changes in peripheral pain-processing; and 2) site-dependent differences of central pain regulation.

4.1. Nerve injury-induced plastic changes in adrenergic pain modulation of trigeminal and spinal nociception

Under normal physiological conditions, peripheral noradrenaline has very little influence on pain (Ali et al., 2000; Davis et al., 1991; Fuchs et al., 2001; Torebjork et al., 1995), and administration of norepinephrine in the spinal cord produces a dose-dependent anti-nociceptive effect (Howe et al., 1983; Reddy et al., 1980). Noradrenaline depresses glutamate release from primary sensory afferent fibers *via* α_2-adrenoceptor-

mediated action on presynaptic terminals (kamisaki et al., 1993; Kawasaki et al., 2003; Pan et al., 1999). The α_2-adrenoceptor agonist decreased post-synaptic responses evoked by dorsal root stimulation in the spinal dorsal horn (North et al., 1984; Sonohata et al., 2004), and activation of spinal α_2-adrenoceptors has been shown to lead to behavioral anti-nociception (Asano et al., 2000; Fisher B et al., 1991; Post et al., 1987; Reddy et al., 1980; Solomon et al., 1989; Takano et al., 1992). Noradrenaline enhances GABAergic synaptic transmission in the spinal dorsal horn *via* activation of the α_1-adrenoceptor (Baba et al., 2000a, 2000b; Gassner et al., 2009; Yuan et al., 2009), and pharmacological activation of α_1-adrenoceptors has been shown to induce behavioral anti-nociception (Aran and Proudfit, 1990; Howe et al., 1983; Orii et al., 2002; Reddy et al., 1980; Tasker et al., 1992). Spinal nerve injury triggers ectopic sprouting of sympathetic nerve fibers within dorsal root ganglion (Chung et al., 1993; McLachlan et al., 1993; Ramer and Bisby, 1997). Following nerve injury, sympathetic fibers migrate and branch into the upper skin dermis (Yen et al., 2006). In addition, afferent nerve fibers become sensitive to sympathetic stimulation and adrenergic compounds (Devor et al., 1994; Korenman and Devor, 1981; Zhang et al., 1997). Administration of noradrenaline or adrenergic compounds in inflamed or neuropathic skin will further aggravate hyperalgesia (Ali et al., 2000; Choi and Rowbotham, 1997; Davis et al., 1991; Drummond, 2009; Torebjork et al., 1995). Post-traumatic interactions between sympathetic and sensory nerves play a role in the development of sensory hypersensitivity, and α_2-adrenoceptors induce excitation of primary afferent neurons following spinal nerve injury (Chen et al., 1996; Leem et al., 1997; O'halloran and Perl, 1997; Zhang et al., 1997). In contrast, α_2-adrenoceptors suppress primary afferent action on pre-synapticterminals in the spinal dorsal horn (Kamisaki et al., 1993; Kawasaki et al., 2003; Pan et al., 2002). Primary sensory afferents receive two different modulations following nerve injury. Noradrenaline-induced and nerve injury-induced hypersensitivities are attenuated by peripheral injection of an α_2-adrenoceptor antagonist (Banik et al., 2001; Tracey et al., 1995). Antagonist studies have demonstrated endogenous ligation action. In endogenous modulation following nerve injury-induced plastic changes, it is thought that the α_2-adrenoceptor facilitates primary sensory afferent excitation, rather than inhibition. In contrast, peripheral administration of an α_2-adrenoceptor agonist attenuates hypersensitivity in inflammatory and neuropathic conditions (Eisenach et al., 2005; Laband'homme et al., 2002; Wei and Pertovaara, 1997), which suggests that exogenous α_2-adrenoceptor stimulation might inhibit primary sensory afferent action, rather than facilitate it. Peripheral α_1-adrenoceptors facilitate hypersensitivity of peripheral nociceptors following spinal nerve injury (Ali et al., 1999; Lee et al., 1999; Ren et al., 2005; Wang et al., 2004). Peripheral activation of α_1-adrenoceptors

aggravates nerve injury-induced hyperalgesic behavior (Kim et al., 2005; Lee et al., 2000; Tracey et al., 1995), and intrathecal administration of an α_1-adrenoceptor agonist facilitates nerve injury-induced allodynia-like behavior (Yaksh et al., 1995). These results demonstrated that the α_1-adrenoceptor might facilitate primary afferent action following spinal nerve injury (Fig. 4). Noradrenaline hyperpolarizes neurons in the spinal trigeminal nucleus (Grudt et al., 1995), which is mediated by α_2-adrenoceptor activation. Noradrenaline also depolarizes GABA-containing inhibitory interneurons in the spinal trigeminal nucleus (Grudt et al., 1995), which is mediated by α_1-adrenocepotor activation. In addition, noradrenaline regulates descending inhibition of nociceptive responses in the spinal trigeminal nucleus (Cahusac et al., 1995), and intrathecal administration of an α_2-adrenoceptor agonist inhibits trigeminal nociceptive behavior (Wang et al., 2002; Zhang et al., 1998). These studies demonstrate that adrenergic modulation of trigeminal nociception without nerve injury is similar to spinal nerve nociception.

However, trigeminal nerve injury does not induce sprouting of sympathetic nerve fibers in the trigeminal ganglion (Benoliel et al., 2001; Bongenhielm et al., 1999; Grelik et al., 2005). Rather, trigeminal nerve injury leads to scarce and transient sympathetic sprouting into the upper skin dermis of the oro-facial region (Grelik et al., 2005; Ruocco et al., 2000; Yen et al., 2006). The incidence of sympathetically evoked pain syndromes is much less following nerve injuries in the face or jaws, compared with limb injuries (Matthews et al., 1989), although sympathectomy does not affect development of ectopic discharge and mechanical sensitivity following trigeminal nerve injury (Bongenhielm et al., 1998). Results from the present study demonstrated that intrathecal administration of an α_2-adrenoceptor agonist, as well as an α_1-adrenoceptor agonist, attenuated trigeminal nerve injury-induced hyperexcitability. These results suggested that peripheral adrenergic modulation following nerve injury does not aggravate trigeminal neuropathic pain, which is different from spinal nerve neuropathic pain (Fig. 4).

4.2. Site-dependent adrenergic pain modulation in the central nervous system

Adrenergic receptors either facilitate or inhibit pain, depending on the activation site, the type of activated adrenoceptor, and the variation of pain stimulus (Petrovaara et al., 2006). Spinal administration of an α_1-adrenoceptor antagonist was shown to decrease anti-nociception in the tail response, which was produced by microinjection of morphine into the ventrolateral periaqueductal gray, but the α_1-adrenoceptor antagonist enhanced anti-nociception in the foot response (Fang and Proudfit, 1998). In addition, intrathecal administration of α_2-adorenoceptor antagonist reduces anti-nociception in the tail response, but not in the foot response (Fang and Proudfit, 1996). These differences are likely due to the site of primary afferent terminal

fields in tail and foot segments (sacral *vs.* lumbar segments), and pain modulatory action induced by spinal adrenoceptors might vary between the spinal segments (Fang and Proudfit, 1998).

Distension of the descending colon and rectum has been commonly used as a rat model for visceral pain (Danzebrink and Gebhart, 1990; Ness and Gebhart, 1998a; Petrovaara and Karmari, 2003). The primary afferent neurons from the descending colon and rectum are located in the T13-L2 and L6-S2 dorsal root ganglia (Nadelhaft and Booth, 1984; Ness and Gebhart, 1988b). The lateral spinal nucleus does not receive cutaneous primary afferent input, but rather visceral primary afferent input, whose neurons are innervated by spinal terminals expressing α_{2C}-adrenoceptors (Olave and Maxwell, 2004). Anti-nociceptive effects of visceral pain are produced by intrathecal administration of an α_2-adrenoceptor agonist, but not by an α_1-adrenoceptor agonist (Danzebrink and Gebhart, 1990), which could reflect the differences between cutaneous and visceral nociceptive modulation.

The medullar nucleus raphe magnus (NRM) plays a significant role in adrenergic descending regulation of pain (Proudfit, 1988). Activation of α_1-adrenoceptors depolarizes, and α_2-adrenoceptor activation hyperpolarizes rostroventromedial neurons, which promote descending pain inhibition (Bie et al., 2003). Results have shown that when an α_1-adrenoceptor antagonist is microinjected into the NRM, anti-nociception, which was induced by a mu-opioid receptor agonist in the periaqueductal gray, was significantly attenuated. In addition, treatment with an α_2-adrenoceptor agonist into the NRM significantly antagonized the analgesia (Bie et al., 2003). However, NRM microinjection of only α_1-adrenoceptor antagonist or α_2-adrenoceptor agonist without a mu-opioid receptor agonist did not affect pain-related behavior (Bie et al., 2003). These results showed that NRM α_1-adrenoceptors might contribute to anti-nociception, and NRM α_2-adrenoceptors might decrease anti-nociception, only under stress conditions (Petrovaara, 2006).

The medullar lateral reticular nucleus (LRN) also plays an important role in adrenergic descending control (Janss and Gebhart, 1987; Liu and Zhao, 1992). In a rat model of mustard oil-induced hyperalgesia, an α_2-adrenoceptor antagonist microinjected into the LRN resulted in an anti-hyperalgesic effect (Mansikka et al., 1996). In contrast, in non-hyperalgesic animals, microinjection of an α_2-adrenoceptor antagonist in the LRN did not produce anti-nociception (Mansikka and Petrovaara, 1995). It is thought that the α_2-adrenoceptor antagonists induce anti-nociceptive effects *via* action to the LRN following inflammation-induced changes (Mansikka et al., 1996).

The noradrenergic locus coeruleus (LC) in the pons plays a major role in descending modulation of pain *via* action on spinal α_2-adrenoceptors (Jones et al., 1991; Proudfit, 1988). An LC lesion does not result in changes to the baseline nociception, but rather enhances sustained nociception (Tsuruoka and Willis, 1996). One study showed that an α_2-adrenoceptor agonist

injected into the LC selectively increased formalin-induced nociception (Petrovaara et al., 1994), whereas an α_2- adrenoceptor antagonist microinjected into the LC resulted in anti-allodynia in nerve-injured animals (Wei and Petrovaara, 2006). Nerve injury induces tonic activation of pontine α_2- adrenoceptors, which promotes neuropathic hypersensitivity by attenuating descending inhibition (Wei and Petrovaara, 2006)

The medulla and pontine are oppsite to the spine in adrenergic pain modulation in neuropathic animals. The present study showed that the anti-allodynic effects of α_1- and α_2-adrenocepor agonists in the spinal trigeminal nucleus were stronger than other spinal segments or loci in the central nervous system (Fig. 4).

1.1. Anti-allodynic effects of spinal α1- and α2-adrenoceptors in trigeminal neuropathic pain

The present study demonstrated that intrathecal administration of the α_1-adrenoceptor agonist phenylephrine and the α_2-adrenoceptor agonist clonidine induced anti-allodynic effects in a rat model of ION-CCI. These results were not consistent with results obtained from a rat model of neuropathic pain following spinal nerve injury. Peripheral adrenergic modulation following nerve injury did not aggravate trigeminal neuropathic pain, suggesting that the anti-allodynic effects of adrenoceptors in the spinal trigeminal nucleus are stronger than other sites in the central nervous system. These results demonstrated that spinal α_1- and α_2-adrenoceptors play a role in spinal inhibition of trigeminal neuropathic pain in a rat model of ION-CCI.

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Figure legends

Figure 1A: In rats with infraorbital nerve ligation: time course of anti-allodynic effects of the intrathecally administered α_1-adrenoceptor agonist phenylephrine. Mechanical thresholds are expressed as mean ± S.E.M for eight rats in each group. * $P < 0.05$, compared with vehicle (saline) group; # $P < 0.05$ compared with 3 µg-treated group; + $P < 0.05$ compared with 10 µg-treated group.

Figure 1B: In rats with infraorbital nerve ligation: mechanical thresholds after intrathecal administration of α_1-adrenoceptor antagonist (prazosin) or vehicle (50% DMSO). Data are expressed as mean ± S.E.M for eight rats in each group.

Figure 1C: In rats with infraorbital nerve ligation: effects of intrathecal pretreatment with α_1-adrenoceptor antagonist (prazosin) or vehicle (50% DMSO) on anti-allodynic effects of phenylephrine. Percentages of maximal possible area under curves (AUC) are shown in the presence of prazosin (3 or 10 µg). Data are expressed as mean ± S.E.M for eight rats in each group. * $P < 0.05$, compared with vehicle (50% DMSO) + agonist group.

Figure 2A: In rats with infraorbital nerve ligation: time course of anti-allodynic effects of the intrathecally administered α_2-adrenoceptor agonist clonidine. Mechanical thresholds are expressed as mean ± S.E.M for eight rats in each group. * $P < 0.05$, compared with vehicle (saline) group; # $P < 0.05$ compared with 3 µg-treated group; + $P < 0.05$ compared with 10 µg-treated group.

Figure 2B: In rats with infraorbital nerve ligation: mechanical thresholds after intrathecal administration of α_2-adrenoceptor antagonist (yohimbine) or vehicle (50% DMSO). Data are expressed as mean ± S.E.M for eight rats in each group.

Figure 2C: In rats with infraorbital nerve ligation: effects of intrathecal pretreatment with α_2-adrenoceptor antagonist (yohimbine) or vehicle (50% DMSO) on anti-allodynic effects of clonidine. Percentages of maximal possible area under curves (AUC) are shown in the presence of yohimbine (3 or 10 µg). Data are expressed as mean ± S.E.M for eight rats in each group. * $P < 0.05$, compared with vehicle (50% DMSO) + agonist group.

Figure 3: In rats with infraorbital nerve ligation: dose-response curves plot the peak effect of intrathecally administered α_1-adrenoceptor agonist (phenylephrine) and α_2-adrenoceptor agonist (clonidine). Data are expressed as mean ± S.E.M of a percentage of maximal possible effect (% MPE) for eight rats.

Figure 4: A schematic diagram depicting nerve-injury-induced plastic changes in adrenergic pain modulation. Abbreviations: α1, α_1-adrenocetors; α2, α_2-adrenoceptors; GABA, GABAergic neurons; intact, intact conditions; injured, nerve-injured conditions; LC, locus coeruleus; NRM, nucleus raphe magnus; LRN, lateral reticular nucleus; Sp5c, spinal trigeminal nucleus; TG, trigeminal ganglion; DH, spinal dorsal horn, DRG, dorsal root ganglion; ↑, facilitation of nociception; ↓, inhibition of nociception; →, no effects of nociception.

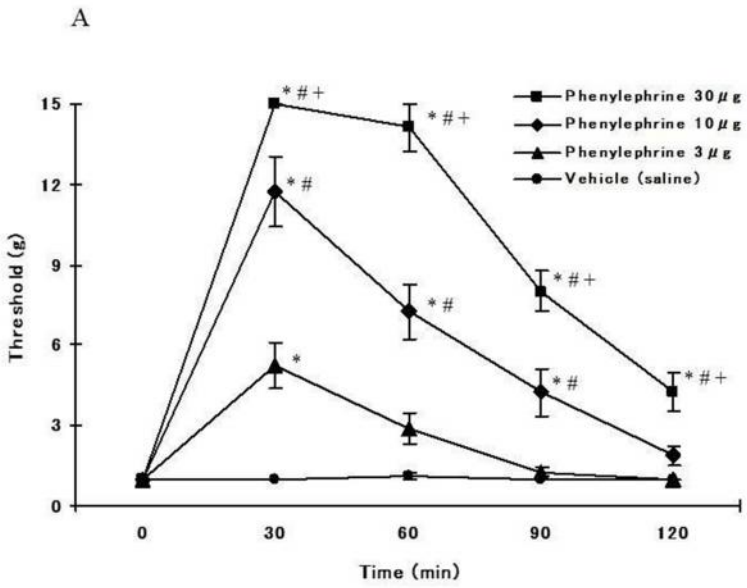
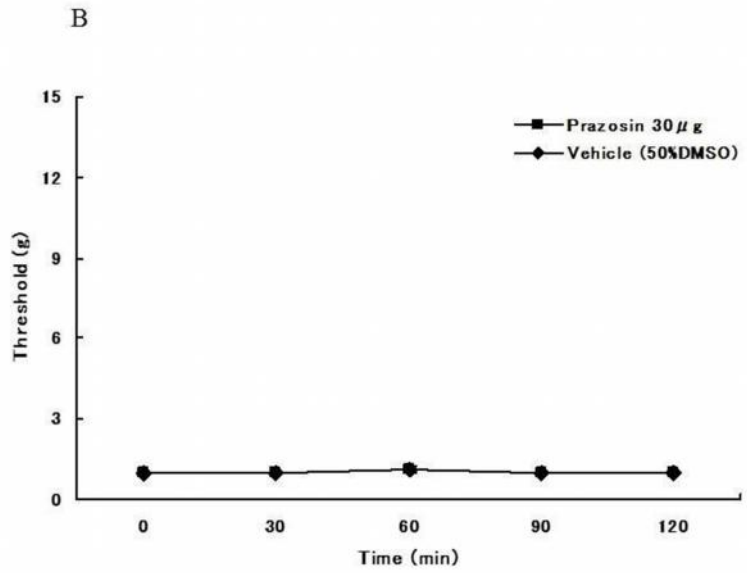


Figure 1A



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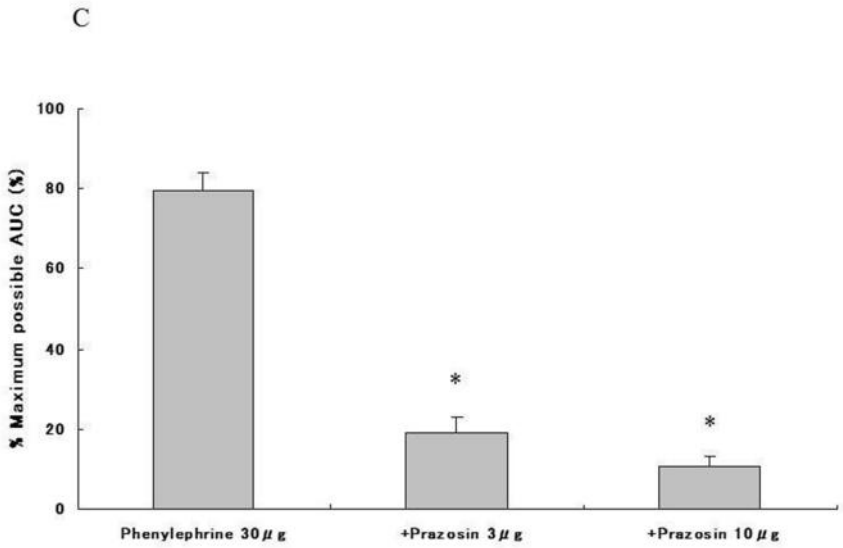

Figure 1C

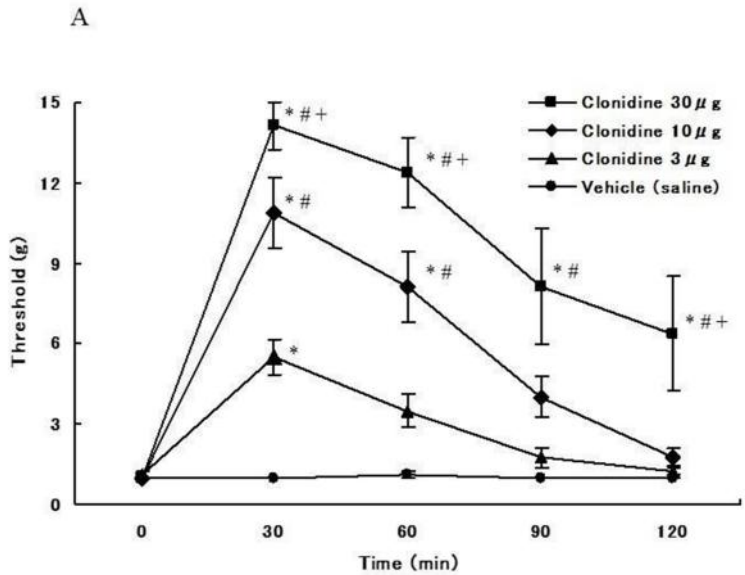

Figure 2A

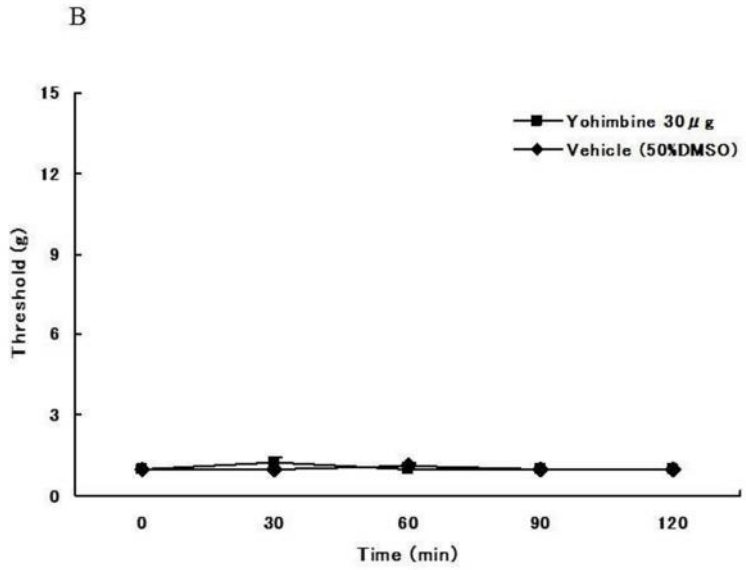

Figure 2B

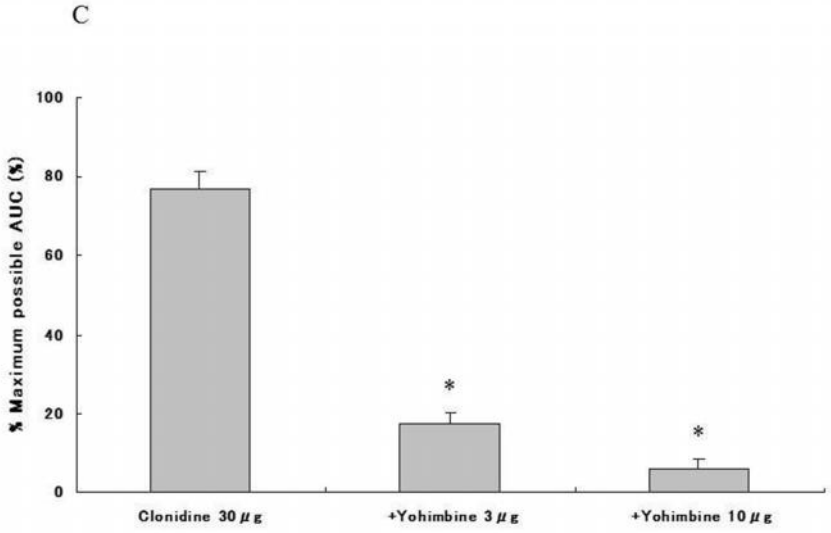

Figure 2C

Anti-allodynic effects following intrathecal administration of α1- and α2-adrenergic receptor agonists in a rat model of trigeminal neuropathic pain

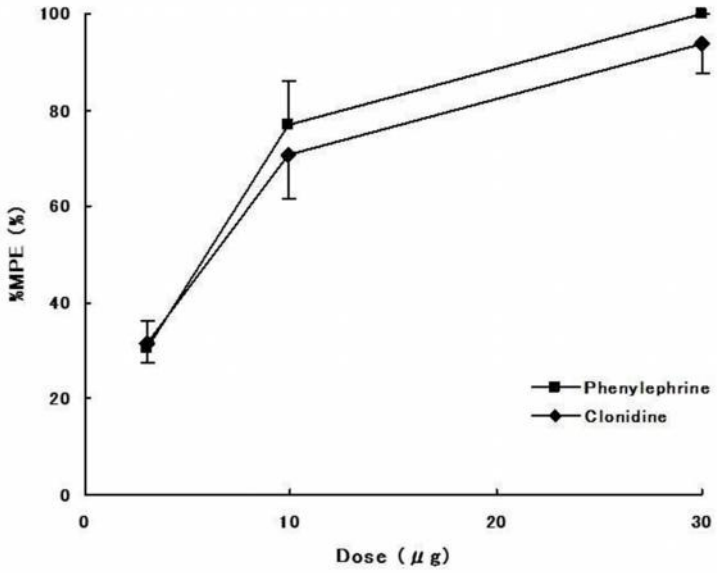

Figure 3

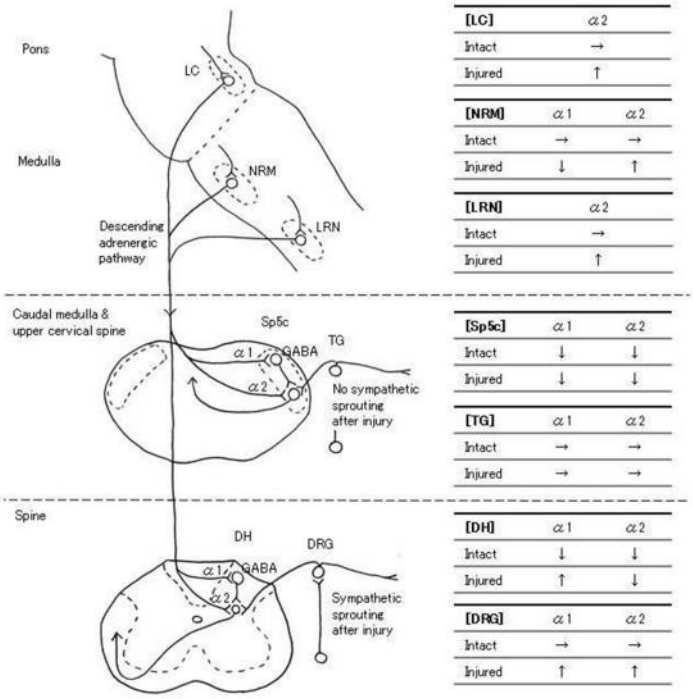

Figure 4