Original

Involvement of a Descending Pathway from the A7 Region in Nociceptive Processing under Neuropathic Conditions in Rats

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Abstract: The A7 cell group in the dorsolateral pons provides noradrenergic innervation of the spinal cord. Activation of this descending pathway (the A7 descending system) produces bi-directional effects on nociceptive processing in the dorsal horn, which are facilitation mediated by α_1 -adrenoceptor and inhibition via α_2 -adrenoceptor. Peripheral nerve injury sometimes results in neuropathic pain. Hypersensitivity of dorsal horn neurons under neuropathic conditions is linked to activity in descending pathways from the brain. The aim of this study was to examine the involvement of the A7 descending system under neuropathic conditions. Experiments were performed on male Sprague-Dawley rats ($n=35$). Bilateral lesions of the A7 area were performed by microinjection of kainic acid. The tibial and common peroneal the nerves were sectioned produce neuropathic conditions (spared nerve injury, SNI). For estimating mechanical allodynia, mechanical hypoalgesia and cold allodynia, paw withdrawal threshold (PWT), paw withdrawal latency (PWL) and paw withdrawal frequency (PWF) were measured. PWTs significantly decreased following A7 lesions. After SNI, PWTs significantly decreased in A7-lesioned and sham-lesioned rats. However, no significant difference was observed between the decreased rates of PWTs in A7-lesioned and sham-lesioned rats. PWLs significantly increased in sham-lesioned rats compared with A7-lesioned group. PWFs significantly increased in the A7-lesioned and sham-lesioned rats. Intrathecal injection of prazosin, an α_1 -adrenoceptor antagonist, failed to change the PWT, PWL and PWF in non-A7-lesioned neuropathic rats. These results suggest that (1) the A7 descending inhibitory system has tonic activity under normal conditions, and (2) this system functions in a complex manner during the neuropathic pain state.

Key words: neuropathic pain, A7 cell group, descending noradrenergic system, pain modulation, spared nerve injury.

Nociceptive sensory inputs receive centrifugal control from the brain, do other sensory inputs. In the spinal cord, nociceptive responses of dorsal horn neurons are modulated by stimulation of many regions of the brain, including the periaqueductal gray, nucleus raphe magnus, nucleus reticularis gigantocellularis, region of the lateral reticular nucleus, rostral ventrolateral medulla, nucleus paragigantocellularis lateralis, parabrachial region, locus coeruleus, subcoeruleus, anterior pretectal nucleus, hypothalamus, thalamic nucleus submedius and other parts of the forebrain (see review by Ref.1)). The A7 catecholamine cell group located in the dorsolateral

pons also has descending noradrenergic projections to the spinal dorsal horn (laminae I-V).²⁾ A7 noradrenergic neurons receive innervation from several brain regions, including the lateral hypothalamus, periaqueductal gray, and rostral ventromedial medulla.^{2~8)} Activation of the descending pathway from the A7 cell group (A7 descending system) produces bi-directional effects on nociceptive processing in the spinal dorsal horn.⁹⁾ These bi-directional effects are facilitation mediated by the α_1 -adrenoceptor⁹⁾ and inhibition via the α_2 -adrenoceptor.^{3,4,9)} The facilitatory and inhibitory modulations of nociceptive inputs are of particular clinical interest because these

modulations may lead to enhancement and reduction of pain, respectively.

Neuropathic pain is a highly debilitating chronic pain state that is a consequence of nerve injury or of diseases such as diabetes, cancer, infection, autoimmune disease, or trauma. Neuropathic pain is often resistant to currently available analgesics. Recent studies have reported that hypersensitivity of dorsal horn neurons under neuropathic conditions is linked to activities in descending pathways from the brain. Reduced activity in descending pathways from either the periaqueductal gray or the locus coeruleus may enhance the nociceptive responses of dorsal horn neurons.^{10~13)} Activation of on-cells in the rostral ventromedial medulla may lead to hypersensitivity of dorsal horn neurons under neuropathic conditions.^{14~16)} Our particular interest is in the role of the A7 descending system under neuropathic conditions because activities in the A7 descending system produce bi-directional effects on nociceptive processing in the spinal dorsal horn. The purpose of the present study was to examine the involvement of the A7 descending system in nociceptive processing under neuropathic conditions. We investigated whether bilateral lesions of the A7 cell group change behavioral responses to either noxious or innocuous mechanical stimuli in neuropathic rats.

Materials and Methods

1. Animals and housing conditions

Adult male Sprague-Dawley rats weighing 200–250 g were housed in plastic chambers with sawdust bedding in a temperature-controlled room (25°C) under a 12 hour light-dark cycle (8 am–8 pm) with food and water available *ad libitum*. Rats were handled 10 min/day for 3 days prior to any experimental manipulation. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Showa University and were in accordance with the International Association for the Study of Pain (IASP) guidelines for pain research on animals.¹⁷⁾

2. Bilateral lesions of the A7 cell group

Rats were placed on a stereotaxic apparatus under pentobarbital anesthesia (Nembutal; 50 mg/kg, *i.p.*). The skull was drilled, and then a microinjection needle connected to a 10 µl Hamilton microsyringe was introduced stereotaxically 8.7 mm caudal to, 2.6 mm lateral to and 7.9 mm below the bregma for the A7 area.¹⁸⁾ Chemical lesioning was performed by microinjection of kainic acid (36 ng dissolved in 100 nl saline over 2 min; Sigma Co.).¹⁹⁾ Following the injection, the needle was left in place for 5 min before being slowly withdrawn. For sham lesions, the tip of the needle was lowered without an infusion. A prophylactic dose of penicillin (20000 i.u., IM) was administered on day of surgery.

3. Spared nerve injury

Under halothane (2%) anesthesia, spared nerve injury (SNI) and sham surgery were performed according to methods described previously.²⁰⁾ After the left sciatic nerve and its three terminal branches were exposed, the common peroneal and tibial nerves were ligated with 5–0 silk. The sural nerve remained intact. In the sham operation, the procedures were the same but the nerves were only exposed and not ligated. Following SNI and sham surgery, muscle and skin were closed in two layers.

4. Nociceptive testing

Rats were tested for behavioral nociception with tactile stimuli, noxious mechanical stimuli and cold stimuli because mechanical allodynia, mechanical hypoalgesia and cold allodynia are typical symptoms in neuropathy. The experiments were carried out during the light phase on independent groups of animals and all testing was conducted in a quiet room by the same person. Rats were placed on an elevated wire mesh grid under an inverted clear plastic chamber (13×13×14 cm). Prior to testing, the rats were kept in the chamber at least 30 min/day for 3 days to acclimatize them to the experimental environment. Behavioral testing was performed on each day with the same sequence of stimuli.

1) Mechanical allodynia

von Frey hairs (VF) were used for testing tactile allodynia. The VF was applied to the plantar surface of the hindpaw. The paw withdrawal threshold (PWT) was taken as the lowest force that evoked a withdrawal response to at least one of five applications, with 15 s intervals between stimuli.²¹⁾ Before the SNI surgery, VF with strengths of 8–26 g were used, whereas after the SNI surgery, hairs with a strength of 1–15 g were used.

2) Mechanical hypoalgesia

The sharp edge of a pin was gently applied to the footpad for mechanical hypoalgesia. The paw withdrawal latency (PWL) was recorded, with a 5 s cut-off. Three measurements were averaged. This test was always preceded by the von Frey hair test.

3) Cold allodynia

Using a syringe connected to PE-90 tubing, a drop of acetone with a diameter of 2 mm was applied to the plantar surface of the hindpaw. Surface tension was used to maintain the volume of the drop at 10–15 μ l. Paw withdrawal response following the application of acetone drop were measured for 1 min. The frequency of paw withdrawal response (paw withdrawal frequency, PWF) in 5 trials at 5 min-intervals was recorded.

4) Drugs

Rats received drug injection under short-term anesthesia by halothane (2%). Prazosin, 30 μ g (Sigma Aldrich, St. Louis, MO, USA), dissolved in 10 μ l saline was intrathecally administered in SNI-induced neuropathic and sham-injured rats. A 27-gauge needle connected to a 50- μ l Hamilton syringe was directly inserted into subarachnoid space of the L5-6 enlargement of the spinal cord through the intervertebral space.²²⁾ The behavioral testing was carried out before and at 30 and 120 min after drug injection. The investigators involved were blinded to the drug given to the animals throughout the experiments.

5) Histology

At the end of the experiments, the animals were deeply anesthetized with sodium pentobarbital (60 mg/kg), and the brain was perfused through the heart with 0.9% NaCl followed by 10% formaldehyde. Frozen serial sections

(50 μ m) were cut and stained with cresyl violet for histological confirmation of the lesion sites.

6) Data analysis

Data are presented as the means $\pm$ S.E.M. Statistical analysis was carried out with analysis of variance (ANOVA) and, with paired *t*-tests for the comparing pre- and post-treatment results. Differences were accepted as significant when $p < 0.05$.

Results

1. Effects of A7 lesions on nociceptive testing

Eleven rats were selected on the basis of histological results confirming that the A7 cell group was almost completely destroyed by bilateral injections of 36 ng of kainic acid. A photograph of lesions of the A7 cell group and a schematic representation of the lesions are shown

Fig. 1 (A) Photomicrograph of a 50- μ m-thick coronal section at the level of the A7 cell group in A7-lesioned rats. The arrow and the circle point to the A7 region. A7 was destroyed by bilateral injection of kainic acid. Cresyl violet-stained coronal sections from the rats treated with kainic acid (3.6 ng). In the A7-treated animals, a marked neuronal cell death and gliosis were observed within the A7 area. (B) Schematic representation of bilateral chemical lesions of the A7 area ($n=11$). The drawing is simplified from.¹⁸⁾

Fig. 2 The effect of bilateral lesions of the A7 region ($n=11$) or sham lesions of A7 ($n=12$) on the paw withdrawal threshold (PWT), response to cold (topical acetone) and response to pinprick on ipsilateral hind paw. Pre, pre-lesions; Post, post-lesions. * $p<0.05$, ** $p<0.01$, significantly different from responses before A7-lesioned rats and between the two groups of animals. Data are represented as the mean $\pm$ SEM.

in Fig. 1A and B, respectively. The location and extent of the lesions were clearly delineated in sections as the area containing reactive neuronal cell death and gliosis within the A7 cell group. In the sham-treated rats, lesions of the A7 cell group were not observed.

The effects of bilateral lesions of the A7 cell group on nociceptive responses are summarized in Fig. 2. Nociceptive testing was performed at 7–10 days after A7

Fig. 3 The time-course of pain-related responses following SNI. SNI induced allodynia and hyperalgesia. Line graph illustrating time-course data collected from the paw ipsilateral to SNI. Tactile allodynia was assessed with both von Frey hairs (A). Cold allodynia was assessed as paw withdrawal response to plantar acetone (B). Mechanical hyperalgesia was assessed as paw withdrawal duration to a pinprick (C) ($n=6$). All data represent the means $\pm$ SEM. The asterisks denote significant differences from the contralateral side; * $p<0.05$.

lesions. In the A7-lesioned rats ($n=11$), PWTs in response to von Frey hairs significantly decreased compared to those before lesions (14.36 ± 1.93 vs. 8.90 ± 0.93 g for pre- and post-lesions, $p=0.003$) (Fig. 2A). PWTs in response to cold stimuli significantly increased (0 vs. 0.54 ± 0.20 times for pre- and post-lesions, respectively, $p=0.02$) (Fig. 2B). PWTs in response to pin prick did not change significantly after A7 lesions (0.43 ± 0.03 vs. 0.34 ± 0.02 s, for pre- and post-lesions, $p=0.07$) (Fig. 2C). In contrast, in the sham-lesioned rats ($n=12$), PWTs, PWTs and PWTs did not significantly differ between pre-

and post-lesion groups (Fig.2A, B and C). In addition, there were significant differences between A7-lesioned and sham-lesioned rats in post-treatment PWTs and PWFs.

2. Effects of neuropathic conditions on nociceptive testing

The time courses of three parameters of nociception following SNI surgery are shown in Fig. 3. In normal rats, SNI significantly reduced PWTs and increased PWFs and PWLs, indicating the presence of tactile allodynia, mechanical hypoalgesia, and cold allodynia, respectively. PWTs for ipsilateral hindpaw were significantly lower than those for the contralateral hindpaw

after post-operative day 3 ($p=0.01$) (Fig. 3A). Rats rarely responded to acetone application before surgery. After the SNI surgery, increased sensitivity to acetone was observed. When acetone was applied to the plantar surface of the hindpaw ipsilateral to the operative side, rats briskly withdrew the foot after some delay (within 1 min) and subsequently shook, tapped or licked it. PWFs were significantly increased for at least 25 days after post-operative day 3 ($p=0.02$) (Fig. 3B). Mechanical hypoalgesia was observed after the SNI surgery. The PWLs peaked 3 days after the surgery, and hypoalgesia was stable from 7 to 28 days (Fig. 3C). No significant change in the three parameters was observed in contralateral hindpaws. In the present study, data

Fig. 4 Histograms summarizing the change in behavior on 7 days after SNI surgery at the ipsilateral and contralateral sides in A7-lesioned rats ($n=6$) and sham lesioned rats ($n=12$). Values represent the means $\pm$ SEM. The asterisks denote significant differences from responses before SNI and between the two groups of rats; $**p<0.01$ and $*p<0.05$.

from day 7 after surgery were used because all three parameters were stable by that time.

Three parameters in nociception after SNI surgery in A7-lesioned rats ($n=6$) and sham-lesioned rats ($n=12$) are summarized in Fig. 4. PWTs significantly decreased in both A7-lesioned and sham-lesioned rats after SNI surgery (Fig. 4A). There was no significant difference in the decrease rate between A7-lesioned and sham-lesioned rats. PWFs significantly increased in both A7-lesioned and sham-lesioned rats after SNI surgery (Fig. 4B); the increase rate in sham-lesioned rats was greater than that in A7-lesioned rats. PWLs significantly increased in both

A7-lesioned and sham-lesioned rats after SNI surgery (Fig. 4C), and the increase rate in sham-lesioned rats was significantly greater than that in A7-lesioned rats. In contrast, no significant change was observed in the contralateral side even after SNI surgery.

3. Effect of intrathecally injected α_1 -adrenoceptor antagonist on neuropathic rats

The potential effects of prazosin, an α_1 -adrenoceptor antagonist, on three parameters in nociception were investigated in non-A7-lesioned neuropathic rats. Prazosin (30 $\mu\text{g}/10 \mu\text{l}$ saline) was administered intra-

Fig. 5 The effect of the α_1 -adrenoceptor agonist injection on the PWT (A), response to cold (B) and prick (C) in SNI rats ($n=6$) and sham experimental neuropathic rats ($n=6$) tested at 14 days after SNI. Data were obtained 30 min and 120 min after i.t. prazosin (30 $\mu\text{g}/10 \mu\text{l}$) for both the ipsilateral (neuropathic side) and contralateral (intact) paws.

thecally into the subarachnoid space at the L5-6 enlargement of the spinal cord, through the intervertebral space. It is known that prazosin effects peak at 20–30 min after administration and then subside to baseline values by 60 min.²³⁾ As shown in Fig. 5, the intrathecal injection of prazosin did not significantly change these three parameters of nociception in neuropathic rats.

Discussion

1. Tonic inhibitory modulation of non-nociceptive sensory inputs by the A7 descending system

It is well known that the A7 descending system has both inhibitory and facilitatory effects on nociceptive processing in the spinal dorsal horn.⁹⁾ In the present study, bilateral lesions of the A7 cell group in normal rats resulted in the change of behavioral responses to innocuous stimuli, the significant decrease of PWTs and the significant increase of PWFs. These results suggest the following: (1) the A7 descending inhibitory system affects not only on nociceptive processing but also non-nociceptive processing in the spinal dorsal horn and (2) the A7 descending inhibitory system is tonically active such that tactile and cold sensory inputs are inhibited under normal conditions. In addition, Dennis and colleagues have shown that the endogenous noradrenergic pain modulation system has only low tonic activity.²⁴⁾ In the present study, however, the PWL nociceptive response was unchanged following A7 lesions, suggesting that nociceptive inputs induced by mechanical noxious stimuli are not subject to tonic inhibition by the A7 descending system. Unfortunately, the neuronal mechanisms of these phenomena are outside the scope of the present study.

2. Involvement of the A7 descending system under neuropathic conditions

As shown in Fig. 4A, PWTs significantly decreased in both A7-lesioned and sham-lesioned rats after SNI surgery. However, no significant differences in the decrease rate were observed between A7-lesioned and sham-lesioned rats. This result suggests that the

A7 descending inhibitory system is not affected by neuropathic conditions.

PWFs were significantly increased in both A7-lesioned and sham-lesioned rats after SNI surgery, but the increased rate was greater in sham-lesioned rats than in A7-lesioned rats. This result shows that cold allodynia is enhanced in sham-lesioned rats. The following two neural phenomena may underlie the SNI-induced enhancement of cold allodynia: suppression of the tonic inhibitory activities of the A7 descending system on cold sensory inputs and activation of facilitatory activities of the A7 descending system. As shown in Fig. 5, intrathecal injection of prazosin, an α_1 -adrenoceptor antagonist, failed to change cold allodynia. Therefore, the mechanism of the enhancement of cold allodynia may be suppression of the A7 descending inhibitory system by SNI.

PWLs were also significantly increased in both A7-lesioned and sham-lesioned rats after SNI surgery. The increase rate was greater in sham-lesioned rats than in A7-lesioned rats. This result indicates that nociceptive hypoalgesia is reduced in sham-lesioned rats. The following two neural phenomena may underlie the reduction of SNI-induced nociceptive hypoalgesia: suppression of the A7 system's tonic inhibition of mechanical nociceptive inputs and activation of facilitatory functions of the A7 descending system. Nociceptive hypoalgesia did not alter when prazosin was intrathecally injected into the spinal dorsal horn. Therefore, the reduction in nociceptive hypoalgesia may be caused by activation of the A7 descending inhibitory system by SNI.

A recent study reported that extensive loss of noradrenergic neurons in the locus coeruleus, A5 and A7 cell groups did not alter nociceptive thresholds but elicited thermal and mechanical hyperalgesia in SNL rats.²⁵⁾ This finding clearly shows that the descending noradrenergic system has little effect on pain in healthy peripheral tissues but changes the balance between facilitation and inhibition of pain following peripheral nerve injury. The results in the present study are consistent with their

findings.

Peripheral nerve injury results in the development of a neuropathic pain state that is characterized by abnormal sensory perception such as allodynia and hyperalgesia.²⁶⁾ It has been confirmed that peripheral nerve injury induces neuroplastic changes in brain regions including the anterior cingulate cortex, insular cortex, amygdala, striatum, thalamus, rostral ventromedial medulla, periaqueductal gray, locus coeruleus, red nucleus, and medulla oblongata.²⁷⁾ The neuroplastic changes in the brain and spinal cord lead to central sensitization and behavioral changes. Neuropathic pain induced by peripheral nerve injury may therefore arise from such neuroplastic changes in the brain and spinal cord.

The A7 cell group receives nociceptive inputs from the spinal lamina I through direct projection²⁸⁾ and from the lateral hypothalamus, which receives nociceptive input from the spinohypothalamic tract.^{2, 5, 29~31)} Thus, the A7 descending system is one component in the feedback loop for modulating nociceptive transmission in the spinal cord.

In conclusion, the present study is the first report to suggest the involvement of the A7 descending system in nociceptive processing under neuropathic conditions. The A7 descending inhibitory system is activated such that nociceptive hypoalgesia is reduced in a neuropathic pain state, although mechanical allodynia is not affected.

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