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Alteration in sensitivity of ionotropic glutamate receptors and tachykinin receptors in spinal cord contribute to development and maintenance of nerve injury-evoked neuropathic pain

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Abstract

Allodynia or hyperalgesia induced by peripheral nerve injury may be involved in changes in the sensitivity of neurotransmitters at the spinal cord level. In order to clarify the functional role of neurotransmitters in peripheral nerve injury, we used rats with nerve injury induced by chronic constriction of the sciatic nerve (CCI rat model) and estimated the effects of the intrathecal injection of drugs known to affect glutamate and tachykinin receptors. In sham-operated rats, the NMDA receptor agonist NMDA and AMPA-kinate receptor agonist *RS*-(5)-bromowillardin reduced withdrawal latency. The non-competitive NMDA receptor antagonist MK-801, competitive NMDA receptor antagonist AP-5 and AMPA-kinate receptor antagonist NBQX increased withdrawal latency. Substance P (SP) increased the withdrawal latency but only transitorily. The NK_1 receptor antagonist RP67580 increased withdrawal latency, but the NK_2 receptor antagonist SR48968 did not show an effect. In CCI rats, *RS*-(5)-bromowillardin reduced withdrawal latency, but NMDA did not show an effect. NBQX increased withdrawal latency, while MK-801 and AP-5 showed little or no effect. SP reduced withdrawal latency, and both RP67580 and SR48968 increased it. These results indicate that the alteration in sensitivity of ionotropic glutamate receptors and tachykinin receptors in the spinal cord contribute to development and maintenance of nerve injury-evoked neuropathic pain.

Keywords: Neuropathy; Thermal hyperalgesia; Neurotransmitters; Glutamate receptors; Tachykinin receptors; Spinal cord; Pain

1. Introduction

It is known that peripheral nerve injury causes changes in the physiology and anatomy of the peripheral and central somatosensory pathways. These changes are associated with chronic neuropathic pain, such as hyperalgesia, allodynia and spontaneous pain, by causing both an increase in the transduction sensitivity of primary afferents (peripheral sensitization) and an increase in the excitability of spinal cord neurons (central sensitization) (Xie and Xiao, 1990; Dubner and Ruda, 1992; Gracely et al., 1992; Kajander and Bennett, 1992).

Within the dorsal spinal cord, both ionotropic glutamate receptors [*N*-methyl-D-aspartic acid (NMDA), γ-amino-3-hydroxy-5-methylis-oxazole-4-propionic acid (AMPA) and kainic acid (KA)] and metabotropic glutamate receptors are involved in nociceptive signaling and central sensitization in conditions of chronic pain (Coderre et al., 1993; Dickenson, 1994; Price et al., 1994; Dickenson et al., 1997). Glutamate functions as a neurotransmitter, and as it is released from presynaptic terminals in an activity-dependent manner in response to excitation between the primary afferent and spinal neurons involved in pain processing; therefore, antagonists of glutamate receptors may therefore be useful as analgesics. This has become evident in studies of NMDA receptor antagonists. Although injection of these drugs in humans reduces many types of acute and chronic pain (Eide et al., 1994; Park et al., 1995; Nelson et al., 1997; Stubhaug et al., 1997), adverse reactions including sedation, dysphoria, catatonia, and sensory distortion limit usable doses to levels where they provide only a modest degree of pain relief. Furthermore, animal and human studies have shown that NMDA antagonists reduce only certain

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components of pain (Tal and Bennett, 1993; Stubhaug et al., 1997), suggesting that non-NMDA receptors or other transmitter systems may also have important functions. Results of animal studies suggest a role in pain processing for the other two classes of glutamate receptors, i.e., AMPA and KA (Kristensen et al., 1992; Nasstrom et al., 1992), as well as for metabotropic receptors (Lutfy et al., 1997), but their analgesic effects have been less intensively studied than those of NMDA antagonists.

Substance P receptors [SP-R, that is neurokinin-1 receptor (NK_1)] are densely distributed in the superficial dorsal horn of the spinal cord where SP-containing primary nociceptive afferents terminate, which suggests that substance P and NK_1 receptors, besides excitatory amino acids and glutamate receptors, play important roles in spinal nociceptive transmission.

The aim of our study was to determine the involvement of glutamate and tachykinin receptors in thermal hyperalgesia following nerve injury. Thermal hyperalgesia was induced by chronic constriction of the sciatic nerve in rats (CCI model), and this model was then used to investigate changes in thermal nociceptive behavior after intrathecal injection of glutamate and tachykinin receptor agonists and antagonists.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (approximately 200–250 g body weight) were used. Food and water were supplied *ad libitum*, and the animals were kept in a 12-h light–dark cycle. At the end of study, rats were euthanized with sodium pentobarbital.

All surgical and experimental procedures were reviewed and approved by the Osaka University Faculty of Dentistry Intramural Animal Care and Use Committee and conform to the guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

2.2. Surgical preparation

2.2.1. CCI surgery

Surgery consisted of a modified version of the model of Bennett and Xie (1998). After the rats had been anesthetized with sodium pentobarbital (50 mg/kg, i.p.), the left sciatic nerve was exposed at mid-thigh level immediately proximal to the point of trifurcation and freed from the surrounding connective tissue. Four chromic gut (4–0) ligatures were tied loosely around the nerve 1–2 mm apart. Sham-operated rats, in which the sciatic nerve was isolated but not ligated, were operated on as controls. In all rats, the contralateral side was left intact.

2.2.2. Intrathecal catheterization

Lumbar intrathecal (i.t.) catheterization was carried out 7 days after CCI surgery by using a modification of the method described by Yaksh and Rudy (1976). After the rats had been anesthetized with sodium pentobarbital (50 mg/kg, i.p.), the dorsal aspect of the L5–L7 vertebra was opened, and the spinous process was removed. A polyethylene catheter (PE10) was introduced into the spinal subarachnoid space approximately 2 cm cranially via the L6 vertebra through a small hole. The hole was made carefully with a dental drill so as not to injure the spinal cord, and the tip of the catheter was placed near the lumbar enlargement of the spinal cord. The other end of the catheter was tunneled under the skin to the cervical region, flushed with saline, and sealed with stainless steel wire. The small hole was sealed with instant adhesive (Alon alfa; Sankyo Co. Ltd., Tokyo, Japan). The catheter was secured to the musculature at the incision, which was then closed. The rats were allowed to recover for a period of at least 7 days prior to testing. All intrathecally injected drugs were given in a volume of 40 μl, followed by a 10 μl saline flush.

2.3. Sensory testing

The Plantar Test (Model 7370; Ugo Basile, Varese, Italy) was used in accordance with previously described methods (Yonehara et al., 1997) to determine whether the rats were hyperalgesic. In brief, prior to testing, the animals were placed in a small cage on a glass plate. They were not restrained and could move about and explore freely. Radiant heat was beamed onto the plantar surface of the hind paw. The intensity of the beam was controlled and adjusted prior to the experiments, and the cutoff latency was set at 24 s.

2.4. Drugs

N-Methyl-D-aspartate (NMDA, Tocris Cookson Ltd., Bristol, UK) was used as NMDA receptor agonist, while dizocilpine maleate (MK-801, Tocris Cookson) and 2-amino-5-phosphono pentanoic acid (AP-5, Tocris Cookson) were used as an NMDA non-selective and NMDA selective antagonist, respectively. *RS*-5-bromowillardin (Tocris Cookson) was used as an AMPA–KA receptor agonist, and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide (NBQX, Tocris Cookson) was used as an AMPA–KA receptor antagonist. Substance P (SP, Sigma Chemical, St. Louis, MO) was used as a sensory neuropeptides. RP-67580 (RP; Rhone-Poulenc Rorer Inc., Collegeville, PA) was used as an NK_1 antagonist and SR48968 (SR; Sanofi Research, Northumberland, UK) as used as an NK_2 antagonist. All drugs were dissolved in 0.9% sterile preservative-free saline, with subsequent dilutions performed in the same manner, with the exception of NBQX, for which a stock solution was prepared using 25 mM bicarbonate (pH 8.0), but subsequent dilutions were made with saline as described above.

2.5. Statistical analyses

Data are presented as the mean values ± standard error of the mean (S.E.M.). The maximum percentage change after drug administration was calculated according to: % change = $100 \times (BA - AA)/BA$, where BA is the withdrawal latency (s) before administration (s) and AA is the withdrawal latency (s) after administration. Maximum percentage changes are shown in the result.

Statistical evaluations were performed with Dunnett's test for multiple comparisons subsequent to analyses of variance (ANOVA). $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Behavioral change

The CCI surgery produced thermal hyperalgesia 3–7 days after loose ligation of the sciatic nerve, and this hyperalgesia continued for 30–40 days (Yoshimura and Yonehara, 2001). No differences were seen between rats with i.t. cannulation before or after nerve ligation (data not shown). After i.t. injection of the sham-operated and CCI rats with saline, withdrawal latency of the ipsilateral and contralateral hind paw remained stable for 5 h during repeated thermal stimulation (Fig. 1). The doses of the drugs used in our study were carefully chosen so as not to induce major changes in general behavior, but occasionally minor general behavioral changes occurred such as sedation and paw licking occurred.

3.2. NMDA receptor agonist and antagonists

Time courses of withdrawal latency (s) after i.t. injection of NMDA (NMDA receptor agonist: 0.1, 1 μM), MK-801 (non-selective NMDA receptor antagonist: 0.5, 5 mM) and AP-5