Electroacupuncture Potentiates the Antiallodynic Effect of Intrathecal Neostigmine in a Rat Model of Neuropathic Pain

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Abstract: This study was performed to examine whether electroacupuncture potentiates the neostigmine-induced antiallodynia in neuropathic pain rats. Although intrathecal neostigmine (0.05, 0.1, and 0.3 μg) dose-dependently relieved cold allodynia, 0.3 μg neostigmine caused side effects. The coapplication of 0.1 μg neostigmine and electroacupuncture, however, produced potent antiallodynia, which was parallel to the effect of 0.3 μg neostigmine, without side effects. These results indicate that electroacupuncture can enhance the antiallodynic action of intrathecal neostigmine.

Key words: intrathecal neostigmine, electroacupuncture, neuropathic pain.

Peripheral nerve injury often results in chronic neuropathic pain, which is characterized by spontaneous burning pain, hyperalgesia, and allodynia. Although numerous studies have attempted to elucidate pathophysiological mechanisms or drug effects for neuropathic pain, the underlying mechanisms are still unclear, and therapeutic outcomes of conventional analgesics have been observed as variable [1–3]. Intrathecally administered cholinesterase inhibitors, such as neostigmine, dose-dependently produces an antiallodynic effect in a rat model of neuropathic pain, with some side effects [4]. In human studies, intrathecal neostigmine also produces side effects with a dose-dependent antinociceptive effect [5, 6]. Therefore it is required to reduce undesirable side effects and to increase the safety of intrathecal neostigmine.

One approach to diminish the incidence or severity of side effects from analgesic therapy is to combine different classes of drugs. Thus it was suggested that a combination of spinal α2-adrenergic stimulation or systemic opiate administration and spinal injection of a cholinesterase inhibitor could enhance analgesia from the former while limiting side effects from spinal cholinergic stimulation [7]. Our previous studies showed antiallodynic effects of electroacupuncture (EA) stimulation in a rat model of neuropathic pain, which are mediated by activation of spinal μ- and δ-opioid receptors, α2-adrenergic receptor, and/or 5-HT1A and 5-HT3 receptors [8, 9]. From these, we hypothesized that a coapplication of intrathecal neostigmine and EA produces a synergistic effect in a rat model of neuropathic pain. To explore this hypothesis, we examined the effects of a combination of intrathecal neostigmine and EA stimulation and both of them independently on cold allodynia, using the rat tail model of neuropathic pain [10, 11].

Young adult male Sprague-Dawley rats ((Sam:TacN(SD))BR, 200–220 g) were housed in groups of four, with water and food available ad libitum. The room was maintained with a 12-h light/dark cycle (08:00–20:00 light, 20:00–08:00 dark) and kept at 23 ± 2°C. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Kyung Hee University.

Neuropathic surgery and behavioral tests were performed as previously described [10, 11]. Briefly, under isoflurane anesthesia the right superior caudal trunk was exposed, freed from the surrounding tissues and transected at the level between the S1 and S2 spinal nerves that innervate the rat tail. The behavioral signs of cold allodynia were sought by immersing the tail in cold water (4°C). Each animal was lightly immobilized in a plastic holder, and its tail was drooped for a proper application of cold.

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water stimuli. Following the tail immersion, the latency to an abrupt tail movement was measured with a cutoff time of 15 s. The tail immersion test was repeated five times at 5 min intervals. When calculating the average latency, the cutoff time was assigned to the normal responses. The average latency was taken as a measure for the severity of cold allodynia; a shorter latency was interpreted as more severe cold allodynia.

Fourteen days after the neuropathic surgery, the rats were implanted under isoflurane anesthesia with PE-10 tubing by a procedure modified from Yaksh [12]. A PE-10 tubing (12.5 cm) that had a knot 8 cm from the tip was inserted into the spinal subarachnoid space through an incision made on the atlanto-occipital membrane. The tip of the tubing lay in the region of the lumbar enlargement, which was examined before actual pharmacological experiments by observing the motor paralysis of the animal’s hindlimb following intrathecal injection of lidocaine and was anatomically confirmed with a methylene blue dye injection after the experiments. The animals were allowed to recover for 5 days. If any signs of the spinal cord or root damage were observed, the animal was discarded.

Neostigmine was obtained from Sigma and dissolved in normal saline (0.9% NaCl). Neostigmine (0.05, 0.1, 0.3, 1, and 3 μg) or vehicle (normal saline) was injected into the intrathecal space in a volume of 10 μl, and the tubing was flushed with 10 μl vehicle after drug injection.

For EA stimulation, a pair of stainless steel acupuncture needles (0.25 mm in diameter and 4 cm long) was inserted into the acupoint “Zusanli” (ST36) on the right hindlimb, which is located in the anterior tibial muscle, 5 mm lateral and distal to the anterior tubercle of the tibia, and into the point 5 mm distal from the first needle. EA stimulation at this point is known to produce antiallodynic effects in a rat model of neuropathic pain [8, 9]. An electrical stimulator was connected to the two acupuncture needles, and train-pulses (2 Hz, 0.5 ms pulse duration, 0.2–0.3 mA) were then applied for 30 min under the restrained condition.

Five days after the implantation of intrathecal catheters, the rats were injected with neostigmine or normal saline. Immediately after the injection, EA was applied to the ST36 for 30 min. Cold allodynia signs were assessed before and from 10 min after the treatments. All behavioral tests were performed under a blind design for the types of treatments.

All data are presented as mean ± SEM. For the statistical analysis, the one-way analysis of variance (ANOVA) followed by a Newman-Keuls multiple comparison test were used.

### Table 1. Incidence of side effects after intrathecal neostigmine and neostigmine-EA combination.

| Dose (μg) | n  | Tremor | Writhing action | Urination |
|-----------|----|--------|-----------------|-----------|
| Neostigmine |     |        |                 |           |
| 0.05  | 7  | –      | –               | –         |
| 0.1   | 7  | –      | –               | –         |
| 0.3   | 7  | 3 (43%)| 1 (14%)         | 2 (29%)   |
| 1     | 4  | 3 (75%)| 3 (75%)         | 2 (50%)   |
| 3     | 2  | 2 (100%)| 2 (100%)       | 2 (100%)  |
| Neostigmine + EA | 7 |        |                 |           |
| 0.1 + EA |    | –      | –               | –         |

n = number of rats.

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**Fig. 1.** Effects of neostigmine and EA stimulation on cold allodynia in a rat model of neuropathic pain. The behavioral signs of cold allodynia were assessed before and after the treatments. Each group, n = 7. Data are presented as mean ± SEM. *p < 0.001, *p < 0.01, *p < 0.05 vs. control; ⋆p < 0.001, ⋆p < 0.01, ⋆p < 0.05 vs. Neo 0.05; ⋆p < 0.05 vs. Neo 0.1; ⋆p < 0.05 vs. EA only by the one-way analysis of variance followed by the Newman-Keuls multiple comparison test.
Following the nerve injury, the rats showed no abrupt tail movement in response to the cold-water stimuli. After the neuropathic surgery, they showed increased sensitivity to cold stimuli. We interpreted this as a sign of cold allodynia. The cold allodynia sign appeared one day after the nerve injury, and maximal allodynia was observed in the second week [9, 10].

The relieving effects of intrathecal neostigmine and EA stimulation on cold allodynia are shown in Fig. 1. Intrathecal neostigmine (0.05, 0.1, 0.3 μg) produced significant antiallodynic effect in a dose-dependent manner, whereas the control group showed no change. The maximal relieving effect of intrathecal neostigmine on cold allodynia was seen at 20 min after injection and gradually decreased. The EA-only group also showed statistically significant increases in response latency for up to 50 min, and this effect was equivalent to 0.1 μg of neostigmine. As in previously reported trials [8, 9], the rats that underwent the same procedure as the EA-only group without the electrical stimulation (i.e., needle insertion only) showed no significant change in response latency at all time points (data not shown). The combination of intrathecal neostigmine (0.1 μg) and EA stimulation produced a synergistic effect on cold allodynia. The antiallodynic effect lasted for more than 80 min, becoming maximal at 20 min, the same as 0.3 μg of neostigmine.

The occurrence of side effects by intrathecal neostigmine is shown in Table 1. Intrathecal neostigmine at doses of 0.3, 1, and 3 μg caused tremor, writhing action, or urination in some rats. However, no side effects were observed in the low doses of neostigmine (0.05, 0.1 μg) groups or in the 0.1 μg neostigmine + EA group.

Intrathecal administration of cholinergic receptor agonists or cholinesterase inhibitors (e.g., neostigmine and edrophonium) produces an antinociceptive effect in animals as well as in humans, which is mediated by spinal muscarinic receptors [5, 6, 13, 14]. Previous studies have shown that intrathecally administered neostigmine has a relieving effect on mechanical allodynia in a rat model of neuropathic pain, which is mediated by the activation of spinal opioidergic and/or α2-adrenergic receptors [8, 9, 18, 19]. From these it can be inferred that the combination of intrathecal neostigmine and EA stimulation produces a synergistic effect and reduces the side effects in a rat model of neuropathic pain. In the present study, EA stimulation significantly relieved the cold allodynia with no side effects, and the efficacy was similar to the antiallodynic action of 0.1 μg neostigmine. Furthermore, the combination of 0.1 μg neostigmine + EA stimulation resulted in a better antiallodynia with no apparent side effects than 0.1 μg neostigmine or EA alone, which were equivalent to the efficacy of 0.3 μg neostigmine. These results suggest that EA stimulation indeed potentiates the antiallodynic effect of a low dose of intrathecal neostigmine without side effects. Since it is still unclear whether the present finding results from the functional interaction between intrathecal neostigmine and EA stimulation or just from the addition of independent actions of the two treatments, further studies are needed to clarify this issue.

In conclusion, the intrathecal administration of neostigmine has a relieving effect on cold allodynia, whereas it causes side effects in a dose-related fashion. The combination of EA stimulation and intrathecal neostigmine at a low dose results in a synergistic antiallodynic effect without apparent side effects in a rat model of neuropathic pain. These findings provide clinically useful evidence for applying neostigmine and EA in the management of neuropathic pain.

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