Itch is a common complaint among patients with cutaneous diseases. H1 histamine receptor antagonists are the drugs of choice for the treatment of itch, but their effect in ameliorating many pruritic diseases including atopic dermatitis is often unsatisfactory. The reason for this may be due in part to the presence of many itch mediators other than histamine (1), and therefore specific antagonists and inhibitors may not effectively relieve many kinds of pruritic diseases. Although at least two subpopulations of primary afferents (histamine sensitive and insensitive) are involved in itch signaling (2), the types of neurotransmitters involved in itch signaling in the dorsal horn may be far fewer than those of itch mediators in the skin. It was recently reported that gastrin-releasing peptide plays an important role in itch signaling from sensory neurons to the interneurons in the dorsal horn (3). Ablation of dorsal horn neurons expressing gastrin-releasing peptide receptor has been shown to result in the suppression of itch-related responses to histamine and several other itch mediators (3). This raises the possibility that administration of antipruritic agents that act on the dorsal horn will be effective for the treatment of itch of many pruritic diseases.

We have recently found that clonidine suppresses the itch-related response of mice to an intradermal injection of serotonin through its action on the α2-adrenoceptors in the spinal cord (4). In mice, cyproheptadine, a serotonin and histamine antagonist, inhibits the itch-related response to serotonin, but does not inhibit the response to mosquito allergy (5, 6). Acute topical application of tacrolimus inhibits itch-related responses to mosquito allergy and proteinase-activated receptor-2 (PAR2) agonist, but does not inhibit the responses to serotonin and histamine (7). Itch signals induced by mosquito allergy may be mediated by primary afferents expressing PAR2 receptors (8). These findings taken together suggest that itch signals of mosquito allergy and serotonin are mediated by separate primary afferents. Therefore, in order to determine whether the stimulation of α2-adrenoceptor in the spinal cord would suppress itch induced by different causes, we investigated whether intrathecal clonidine would suppress the itch-related response to mosquito allergy. The descending noradrenergic system exerts tonic inhibition on pain transmission in the dorsal horn mediated by α-adrenoceptors (9, 10). Thus, we also investigated whether the descending monoaminergic systems exert a tonic inhibition on the transmission of itch signals in the spinal cord.

Male ICR mice (6 – 12-weeks-old; Japan SLC, Shizuoka) were used. They were kept in a room under controlled temperature (22 ± 1°C), humidity (55 ± 10%), and light (light on 7:00 – 19:00 h). Food and water were

**Keywords**: allergic itch, clonidine, descending noradrenergic system

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**Abstract.** We investigated whether the descending noradrenergic system regulates allergic itch. Mosquito allergy of the hind paw elicited biting, an itch-related response, in sensitized mice. The biting was inhibited by intrathecal clonidine and reversed by yohimbine, an α2-adrenoceptor antagonist. The biting was increased by intrathecal pretreatment with the catecholaminergic neurotoxin 6-hydroxydopamine and the α-adrenoceptor antagonist phentolamine but not the serotonergic neurotoxin 5,7-dihydroxytryptamine. We propose that α2-adrenoceptors are involved in the inhibition of allergic itch in the spinal cord and that the descending noradrenergic system exerts a tonic inhibition on the itch signaling. The serotonergic system may not be involved.

**Keywords**: allergic itch, clonidine, descending noradrenergic system
freely available. Experiments were conducted with the approval of the Committee for Animal Experiments at University of Toyama and according to the guiding principles for the care and use of laboratory animals approved by The Japanese Pharmacological Society.

Mosquito allergen was prepared from the thorax including the salivary gland of female mosquitoes (Aedes albopictus) and dissolved in physiological saline before use. The 10-μg dose of mosquito allergen was injected intradermally into the caudal back twice a week for 4 weeks for sensitization and into the plantar region of the hind paw 7 days later for challenge; the injection volume was 50 and 20 μl for sensitization and challenge, respectively.

Naloxone hydrochloride was dissolved in physiological saline and injected subcutaneously 15 min before challenge. Clonidine hydrochloride, yohimbine hydrochloride, and phentolamine hydrochloride were dissolved in distilled water and injected intrathecally 5 min before challenge. 6-Hydroxydopamine (6-OHDA) hydrochloride and 5,7-dihydroxytryptamine (5,7-DHT) creatinine sulfate were dissolved in physiological saline containing 0.1% ascorbic acid. 6-OHDA (20 μg/mouse) and 5,7-DHT (60 μg/mouse) were injected intrathecally 3 days before challenge; desipramine hydrochloride (25 mg/kg) was injected intrathecally 30 min before the 5,7-DHT injection to block its uptake into the noradrenergic nerve terminals. All agents were purchased from Sigma (St. Louis, MO, USA) and their weights refer to the salts. Intrathecal injection was performed in unanesthetized animals in a volume of 5 μl through a lumbar puncture.

The biting of the pruritogen-treated hind paw was observed as an index of itch. Before behavioral observations, the mice were individually placed in an acrylic cage for acclimation for at least 30 min. The cage was comprised of four equal-sized cells (13 × 9 × 35 cm) with a transparent acrylic floor. Immediately after challenge, the mice were returned to the same cells, and their behaviors were videotaped from below the cage for 30 min; during this period, personnel remained outside the observation room. The video-tape was played back to observe behaviors. The amount of time an animal spent biting the injected site was measured. Licking and claw biting following scratching were not measured.

All data are presented as mean and S.E.M. Results were analyzed with Student’s t-test or Dunnett’s multiple comparisons; P < 0.05 was considered significant.

A challenge with mosquito allergen markedly elicited the biting of the injected site in sensitized mice. The effect peaked during the second 5-min period and almost completely subsided 30 min after the injection (Fig. 1A). The cumulative time spent biting was significantly longer than that of the vehicle-treated group (Fig. 1B). The time course was similar to that reported previously and shorter than the time course of scratching measured after the challenge of the rostral back with mosquito allergen (11). Allergen-induced biting was significantly suppressed by subcutaneous administration of naloxone (1 mg/kg) (Fig. 1C). Scratching elicited by mosquito allergen was also suppressed by naloxone (11). Opioid antagonist may inhibit itch-related responses through the action on the lower brainstem, but not on the spinal cord (14). The present result supports the idea that the biting response to pruritic stimulation is an itch-related behavior.

Intrathecal injection of clonidine (0.1 μg/site) was found to produce a significant 71.6% inhibition in the biting response to allergen challenge, and the inhibition was antagonized by the α2-adrenoceptor antagonist yohimbine (Fig. 2). The results were similar to the biting response to serotonin stimulation, which was markedly suppressed by intrathecal clonidine (0.1 μg/site) and reversed by simultaneous administration of yohimbine (4). Serotonin-induced biting is also inhibited by clonidine at
intraperitoneal doses of 3 and 10 μg/kg, and the inhibitory action of the 3 μg/kg dose of clonidine is reversed by intrathecal yohimbine (4). On the other hand, intracisternal and intraplantar injections of clonidine do not inhibit serotonin-induced biting (4). With these findings taken into account, the present results suggest that the stimulation of α₂-adrenoceptors in the spinal cord relieves itch induced by immediate allergy. With regard to pain, intrathecal injections of clonidine (2.7 – 270 μg/site) produce partial inhibition of the pain-related response to heat stimulation (15). Thus, the antipruritic dose of intrathecal clonidine is lower than the analgesic dose and clonidine may relieve pruritus with fewer side effects.

Only 10% (8/81) of nociceptive neurons in the dorsal horn respond to allergic itch stimulation (12) and itch-signaling neurons are primarily localized in the superficial layers (3, 8, 12). These findings could provide an explanation for the potent anti-pruritic effect of intrathecal clonidine.

The biting response to challenge with mosquito allergen was found to be significantly increased by intrathecal pretreatment with 6-OHDA (20 μg/site), but was not affected by intrathecal 5,7-DHT (60 μg/site) (Fig. 3A). The same doses of 6-OHDA and 5,7-DHT were shown to produce selective depletion of noradrenaline and serotonin, respectively, from the spinal cord (13). Thus, the present results suggest that the descending noradrenergic system exerts tonic inhibition on allergic itch signaling in the spinal cord. The serotonergic system does not appear to be involved. Intrathecal injection of the α₁-adrenoceptor antagonist phentolamine (0.3 and 1.0 μg/site) was found to produce a dose-dependent increase in the biting response to the challenge (Fig. 3B), confirming the tonic noradrenergic inhibition of allergic itch signaling. Intrathecal injection of phentolamine, but not the serotonin antagonist methysergide, increases aversive responses to noxious (algesic) stimulation (10). Thus, the descending monoaminergic systems may exert similar regulation on itch and pain signaling in the spinal cord. We do not exclude the possibility that itch signaling is suppressed by the stimulation of serotonergic receptors in the spinal cord. In a preliminary study, we found that the biting response of naive mice to an intradermal injection of serotonin was also increased by intrathecal pretreatment with 6-OHDA but not 5,7-DHT. In addition, intrathecal clonidine (0.1 μg/site) produced a similar (about 70%) reduction in biting responses to mosquito allergy (present experiment) and intradermal serotonin (4). Thus, the activity of the descending noradrenergic and serotonergic systems might not be substantially affected by sensitization to mosquito allergens.

In summary, the present results suggest that the stimu-
lation of $\alpha_2$-adrenoceptors in the spinal cord inhibits itch elicited by different causes. The descending noradrenergic system may exert tonic inhibition of itch signaling in the spinal cord, and therefore it is possible that pruritus is relieved by antidepressants that block the reuptake of noradrenaline.

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