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Clonidine inhibits itch-related response through stimulation of α_2-adrenoceptors in the spinal cord in mice

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ABSTRACT

The present study investigated whether clonidine – an α_2-adrenoceptor agonist known to relieve pain – is able to suppress itch-related behavior in mice. An intraplantar injection of serotonin induced biting (an itch-related response), which was inhibited by intraperitoneal and intrathecal, but not intraplantar or intracisternal, clonidine injections. The effect of intrathecal clonidine was inhibited by intrathecal injections of phentolamine (a non-selective α-adrenoceptor antagonist) and yohimbine (a selective α_2-adrenoceptor antagonist), but not by prazosin (a selective α_1-adrenoceptor antagonist). The effect of intraperitoneal clonidine was also inhibited by intrathecal yohimbine. These results suggest that clonidine is an effective antipruritic agent and that the effect is mainly mediated by the stimulation of α_2-adrenoceptors in the dorsal horn.

1. Introduction

Itch (or pruritus) is the most common symptom of cutaneous disease (e.g., atopic dermatitis) and systemic disorders (e.g., chronic renal failure). H_1 histamine receptor antagonists are the drug of choice for the treatment of itching, but many pruritic diseases (except acute urticaria) respond poorly to these agents (Krause and Shuster, 1983; Wahlgren et al., 1990). The reason for this may be in part due to the presence of itch mediators other than histamine. Since there are many endogenous itch mediators, including serotonin, substance P, leukotriene B_4, tryptase, and nitric oxide (Andoh and Kuraishi, 1998, 2003; Andoh et al., 1998; Ui et al., 2006; Yamaguchi et al., 1999), specific antagonists and inhibitors may be unable to effectively relieve many kinds of pruritic diseases.

Although at least two subpopulations of primary afferents are involved in itch signaling (Davidson et al., 2007; Johanek et al., 2007; Nakano et al., 2008), there may be far fewer kinds of neurotransmitters involved itch signaling in the dorsal horn than itch mediators in the skin. Therefore, antipruritic agents that act mainly on the dorsal horn may be more effective for the treatment of pruritic diseases than peripherally acting antipruritic agents. There are two case reports that demonstrate that oral and epidural administration of clonidine – an α_2-adrenoceptor agonist – reduces itch (Elkersh et al., 2003; Schwartz and Rosenfeld, 1993). Clonidine has been shown to relieve pain mainly through its action on the dorsal horn (for review, *see* Philipp et al., 2002). Therefore, we investigated whether clonidine is an effective antipruritic in animal experiments.

Serotonin is an efficient endogenous pruritogen in mice (Hagiwara et al., 1999; Yamaguchi et al., 1999; Maekawa et al., 2000). This monoamine elicits hind paw scratching when injected into the rostral back (Yamaguchi et al., 1999; Maekawa et al., 2000), whereas it elicits biting and licking when injected into the hind paw (Hagiwara et al., 1999). The opioid antagonist, naloxone, inhibits biting as well as scratching. However, it does not inhibit licking, which is a pain-related behavior (Hagiwara et al., 1999; Yamaguchi et al., 1999). Opioid antagonists suppress itching and scratching in patients with pruritic diseases (for review, *see* Twycross et al., 2003) and scratching in animal models of various kinds of itch (Andoh et al., 1998; Ohtsuka et al., 2001; Yamaguchi et al., 2001; Miyamoto et al., 2002). The antipruritic effects of opioid antagonists are mediated mainly by central action (Maekawa et al., 2002; Nojima et al., 2003; Andoh et al., 2008; Kuraishi et al., 2008). Taken together, these findings suggest that the biting behavior elicited by intraplantar serotonin is an itch-related response in mice. Biting of the hind paw is useful for testing the central site (brain and/or spinal cord) of the antipruritic action of agents (Kuraishi et al., 2008). Thus, in this study, we used serotonin-induced biting behavior as an index of itch to evaluate the antipruritic effect and to determine the site of action of clonidine.

2. Materials and methods

2.1. Animals

Male ICR mice (Japan SLC, Shizuoka, Japan) that were 6–12 weeks old were used. They were kept under controlled temperature (22 ±

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1 °C) and humidity (55 ± 10%). Their environment was lit from 7:00 AM to 7:00 PM. Food and water were freely available. The study was approved by the Committee for Animal Experiments at the University of Toyama.

2.2. Agents and administration

Serotonin hydrochloride was purchased from Wako Pure Chemical Industries (Osaka, Japan). Clonidine hydrochloride, phentolamine hydrochloride, prazosin hydrochloride, and yohimbine hydrochloride were purchased from Sigma (St. Louis, MO, USA). Serotonin was dissolved in physiological saline, and 20 μl of solution was injected into the plantar aspect of the hind paw. Clonidine was dissolved in physiological saline or distilled water. Phentolamine, prazosin, and yohimbine were dissolved in distilled water. Intrathecal and intracisternal injections were performed in unanesthetized animals in a volume of 5 μl according to the methods of Hylden and Wilcox (1980) and Ueda et al. (1979), respectively. Intraperitoneal, intrathecal, and intracisternal injections were performed 30, 5 and 5 min before serotonin injection. For intraplantar administration of clonidine, it was injected together with serotonin. Weights of drugs refer to the salts.

2.3. Behavioral experiments

Biting of the pruritogen-treated hind paw was observed as an index of itch as described by Hagiwara et al. (1999). Before behavioral observation, the mice were individually placed in an acrylic cage comprised of four equal-sized cells (13 × 9 × 35 cm) with a transparent acrylic floor for at least 30 min for acclimation. Immediately after injection into the plantar aspect of the hind paw, the mice were returned to the same cells, and their behaviors were videotaped from below for 30 min; during this period, personnel remained outside the observation room. The video tape was played back to observe behaviors. Serotonin injection elicited biting (itch-related behavior) and licking (pain-related behavior) and the amount of time an animal spent biting the injected site was measured; licking and claw biting following scratching were not measured. Since the observers could know the treatment which was recorded on a video tape, the experiments were done in a single blind manner.

The sedative effects of clonidine were assessed using a running wheel as previously described (Sasaki et al., 2003). Mice were subject to daily exercise for 3 days by exposure to a voluntary running wheel (25 cm in diameter and 6-cm-wide; Melquest, Toyama, Japan). The next day, the mice were given clonidine and immediately placed in the same running wheel. The number of rotations was automatically counted for 30 min from 5 min after injection.

2.4. Data analysis

Data are presented as means and S.E.M. Statistical significance was determined using Dunnett's multiple comparisons or Student's *t*-test; $P<0.05$ was considered significant.

3. Results

3.1. Effects of systemic injection of clonidine on serotonin-induced biting

An intraplantar injection of serotonin at a dose of 100 nmol/site – the maximal effective dose (Hagiwara et al., 1999) – induced biting of the injected hind paw, although injection of the vehicle (saline) elicited only very slight biting (Fig. 1A). The effect peaked during the second 5-min period and almost completely subsided by 30 min (Fig. 1B).

Intraperitoneal injections of clonidine (1–10 μg/kg) produced a dose-dependent inhibition of biting induced by serotonin (100 nmol/site); significant inhibition was observed at doses of 3 μg/kg or more, with almost complete inhibition at 10 μg/kg (Fig. 2). At an intraperitoneal dose of 10 μg/kg, clonidine did not affect locomotor activity, which was determined using a wheel cage; relative locomotor activity (%) was 100.0 ± 4.5 and 101.8 ± 8.2 ($n=6$ each) in the vehicle- and clonidine-treated groups, respectively.

3.2. Site of inhibitory action of clonidine

Intrathecal injections of clonidine (0.03–0.3 μg/site) produced a dose-dependent inhibition of biting induced by serotonin (100 nmol/

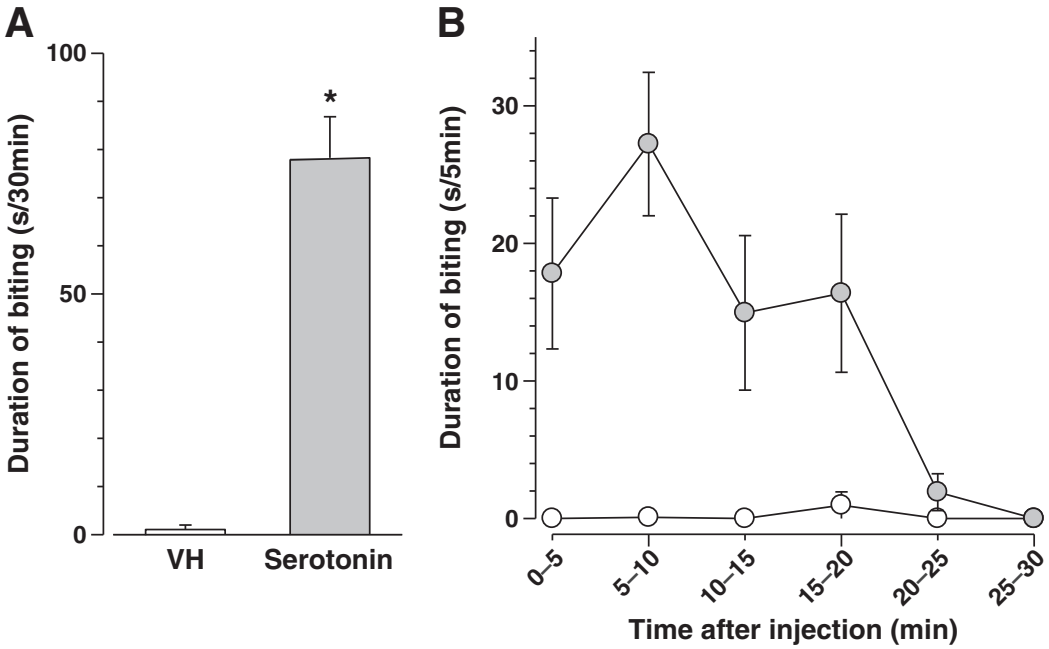

Fig. 1. Serotonin-induced biting response in mice. Serotonin (100 nmol/site) or vehicle (VH, saline) was injected subcutaneously into the plantar region of the hind paw. (A) Total time spent biting the treated paw. (B) Time course of biting following saline (open circles) or serotonin (filled circles) injection. Values represent the mean and S.E.M. $n=6$ (VH), $n=8$ (serotonin). $^*P<0.05$ compared to the VH (Student's *t*-test).