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Ethanol aggravates itch-related scratching in hairless mice developing atopic dermatitis

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ABSTRACT

In patients with atopic dermatitis, alcoholic beverages can sometimes trigger or enhance itching. We have previously reported that HR-1 hairless mice fed a *commercial* special diet, HR-AD, but not a normal diet, develop atopic dermatitis-like skin inflammation with prolonged spontaneous scratching, and that skin barrier dysfunction is involved in the basal scratching. In the present study, the effects of ethanol on itch-related scratching were examined in this mouse model. When ethanol (30%, 10 ml/kg) was given orally to HR-AD-fed mice, scratching with long duration was further markedly increased, while oral ethanol administration had little effect on the scratching response in normal diet-fed mice. The scratching response after oral ethanol administration in HR-AD-fed mice (ethanol-induced scratching) was attenuated by antagonism of the μ-opioid receptor or local skin anesthesia, as in human itching. Ethanol-induced scratching was also suppressed by improvement of skin barrier function by an application of petrolatum ointment, while ethanol administration itself did not affect the function. This suggests that ethanol indirectly aggravates the basal scratching. Although antagonism of the transient receptor potential vanilloid-1 did not affect ethanol-induced scratching, blockade of ethanol actions in the central nervous system (CNS), including γ-aminobutyric acid type A receptor antagonism and *N*-methyl-D-aspartate receptor activation, inhibited it. Taken together, the present study demonstrates that orally administered ethanol markedly aggravates itch-related scratching in HR-AD-fed mice developing atopic dermatitis, and suggests that the CNS depressant actions of ethanol play an important role in the aggravation.

1. Introduction

Atopic dermatitis is a chronic, relapsing, inflammatory skin disease that is increasing in prevalence (Williams and Flohr, 2006). The most common symptom in atopic dermatitis is persistent itching, which severely impairs the patient's quality of life (Koblenzer, 1999). In addition, scratching caused by the itching worsens the skin inflammation, which in turn triggers further itching (Wahlgren, 1999). Thus, the itch–scratch cycle is involved in the development and maintenance of the disease; however, the precise underlying mechanisms are not fully understood.

In patients with atopic dermatitis, alcoholic beverages can sometimes trigger or enhance itching (Stander and Steinhoff, 2002). It is widely accepted that ethanol, contained in alcoholic beverages, is a depressant of the central nervous system (CNS), and that acute systemic ethanol exposure produces various behavioral effects, including anxiolytic, anticonvulsant, ataxic, and sedative/hypnotic effects. Many lines of evidence suggest important roles for several proteins that can affect neuronal excitability, such as the γ-aminobutyric acid type A ($GABA_A$) receptor and the *N*-methyl-D-aspartate (NMDA) receptor, in the acute actions of ethanol in the CNS (Grobin et al., 1998; Mihic, 1999; Allgaier, 2002; Davies, 2003). On the other hand, it has been reported that ethanol stimulates peptidergic primary sensory neurons in guinea pig airways via the activation of the transient receptor potential vanilloid-1 (TRPV1), which results in local neurogenic inflammation (Trevisani et al., 2004). However, little is available on the relationship between these actions of ethanol and itching perception.

Experimental animal models provide an explanation for the mechanisms of disease development. We have demonstrated that HR-1 hairless mice fed a *commercial* special diet, HR-AD, but not a normal diet, develop atopic dermatitis-like symptoms characterized by severe dry skin, inflammatory cellular infiltration in the skin, and elevation of serum immunoglobulin E levels (Fujii et al., 2005). Analyses of spontaneous scratching behavior revealed that although there was no constant difference in cumulative duration and frequency of scratching bouts between normal diet- and HR-AD-fed mice, the duration of one scratching bout was reproducibly prolonged in HR-AD-fed mice (Fujii et al., 2005, 2006). The spontaneous prolonged scratching was suppressed by μ-opioid receptor antagonism, but not by histamine H_1 receptor antagonism (Fujii et al., 2006), which is consistent with the findings in studies of human atopic dermatitis (Monroe, 1989; Wahlgren et al., 1990; Heyer et al., 1997;

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Rukwied et al., 2000). Furthermore, in HR-AD-fed mice, skin barrier dysfunction was coincident with the scratching changes, and improvement of skin barrier function by an application of petrolatum ointment inhibited the scratching (Fujii et al., 2006). These results suggest that this barrier defect could be involved in the basal scratching in HR-AD-fed mice.

The present study was conducted to examine the effects of ethanol on the itch-scratching response in HR-AD-fed mice. Our findings provide evidence for a linkage between the pharmacological actions of ethanol and perception of the itching sensation, especially in atopic dermatitis.

2. Materials and methods

2.1. Animals and diets

Four-week-old, female, HR-1 hairless mice (Hoshino Experimental Animal Center, Yashio, Japan) were used. As described previously (Fujii et al., 2005), atopic dermatitis-like symptoms were induced by feeding them with a *commercial* special diet developed for the HR-1 mice (HR-AD diet; Nosan Corp., Yokohama, Japan) in a conventional animal room under controlled temperature (22–24 °C) and humidity (50–70%) with light (lights on from 08:00 to 20:00). Tap water was given *ad libitum*. As a negative control, mice were fed a normal diet (F-2; Funabashi Farm, Chiba, Japan) under the same conditions. The detailed ingredients, indicated by the manufacturer of each diet, have been described elsewhere (Fujii et al., 2005).

This animal study was approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

2.2. Drug preparation

Ethanol (Wako Pure Chemical Industries, Osaka, Japan) was diluted in distilled water when orally administered, or in physiological saline when intradermally administered. Naltrexone hydrochloride (Sigma Chemical Co., St. Louis, MO, USA), picrotoxin (Sigma), and NMDA (Sigma) were dissolved in physiological saline. Dibucaine hydrochloride (Sigma) was dissolved in physiological saline, and then the solution was mixed with hydrophilic ointment (Maruishi Pharmaceutical Co., Osaka, Japan). Petrolatum ointment purchased from Nippon Shinyaku Co. (Kyoto, Japan) was used. Capsazepine (Sigma) was dissolved in physiological saline containing 10% (v/v) dimethyl sulfoxide and 10% (v/v) Tween® 80. (+)-Bicuculline (Tokyo Chemical Industry Co., Tokyo, Japan) was dissolved in 0.1 N HCl, and then the pH of the solution was adjusted to approximately 6 with NaOH, followed by diluting in physiological saline. Each drug was prepared immediately before use.

2.3. Histology

The cervical dorsal skin of the mice was removed 8 weeks after the start of feeding for histology. The skin sections were stained with hematoxylin and eosin and assessed under a light microscope.

2.4. Analysis of scratching behavior

Mice were acclimatized for 30 min in a measuring cage, followed by recording of scratching behavior on videotape using a CCD camera for 1 h. One scratching bout by the hind paws was defined as a series of scratching movements that ended when the mouse either licked its hind paw or placed its hind paw back on the floor. Scratching behavior was analyzed by playing back the videotape with a specifically developed apparatus that allows analysis of the scratching behavior based on three parameters: 1) cumulative duration, which is determined by having the observer touch the switch for as long as the scratching behavior is observed; 2) frequency, which is determined by an observer who touches a switch each time scratching is observed; 3) duration of one bout, which is calculated by dividing the cumulative duration by the frequency.

2.5. Drug administration

In all cases of oral administration of ethanol, ethanol solution was given at a volume of 10 ml/kg through a plastic probe. When distilled water and 3%, 10%, and 30% ethanol solution were sequentially administered to the same animal, they were given at 2-h intervals. In the case of intradermal administration of ethanol, physiological saline and 0.01%, 0.1%, and 1% ethanol solution were sequentially injected into the cervical dorsal skin at 2-h intervals.

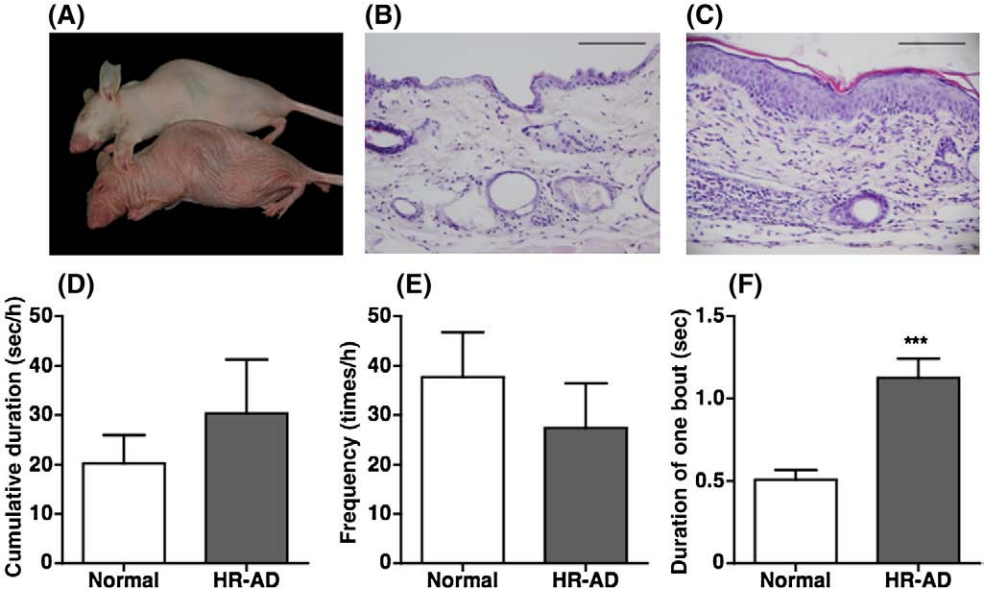

Fig. 1. Development of atopic dermatitis-like symptoms and prolongation of duration of one bout of spontaneous scratching in HR-AD-fed mice. Representative examples of the clinical appearance (A) of normal diet-fed mice (backward) and HR-AD-fed mice (forward), and sections stained with hematoxylin and eosin in normal diet-fed mice (B) and HR-AD-fed mice (C) 8 weeks after the start of feeding. Scale bar represents 100 μm. Cumulative duration (D), frequency (E), and duration of one bout (F) of spontaneous scratching 8 weeks after the start of feeding. Each column represents the mean ± S.E.M. of 5 animals. ***$P<0.001$.

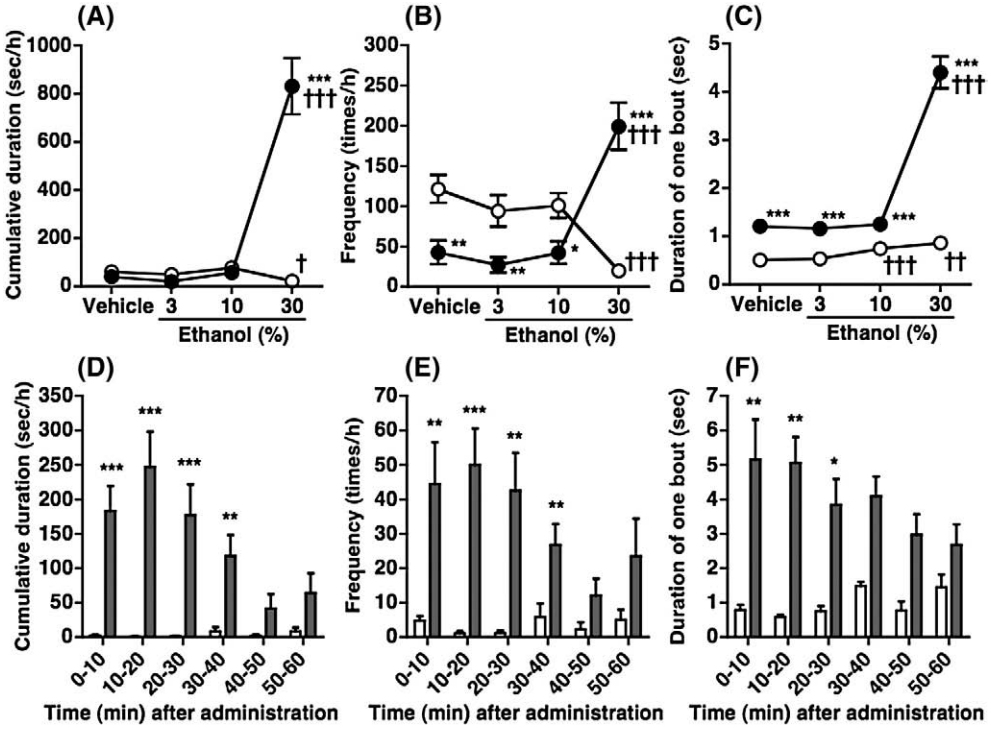

Fig. 2. Aggravation of scratching by orally administered ethanol in HR-AD-fed mice. Cumulative duration (A), frequency (B), and duration of one bout (C) of scratching after oral administration of various doses of ethanol in normal diet- and HR-AD-fed mice 8 weeks after the start of feeding. Distilled water (vehicle, 10 ml/kg) and ethanol solution (3%, 10%, and 30%, 10 ml/kg) were orally and sequentially administered at 2-h intervals. Each point represents the mean ± S.E.M. of 11 or 12 animals. *P<0.05, **P<0.01 and ***P<0.001 vs. normal diet, and †P<0.05, ††P<0.01 and †††P<0.001 vs. vehicle. Time-course changes in cumulative duration (D), frequency (E), and duration of one bout (F) of scratching after the oral administration of the high dose of ethanol (30%, 10 ml/kg) in normal diet- and HR-AD-fed mice. Each column represents the mean ± S.E.M. of 3–12 animals. *P<0.05, **P<0.01 and ***P<0.001.

Effects of drugs on ethanol-induced scratching were assessed based on the response to oral ethanol administration (30%, 10 ml/kg). Naltrexone (10 mg/kg) or capsazepine (100 μmol/kg = 37.7 mg/kg) was subcutaneously administered 30 min before ethanol administration. Dibucaine ointment [0.1 and 1% (w/w)] was applied on the mice's whole skin at a dose of 150 mg/animal 1 h before dosing with ethanol. Petrolatum ointment (200 mg/animal) was applied on the whole skin of mice immediately after ethanol administration. Picrotoxin (1 and 3 mg/kg) or bicuculline (1 and 3 mg/kg) was given intraperitoneally immediately after ethanol administration. NMDA (1.3 and 4 nmol/10 μl/site) was intracisternally administered 10 min after ethanol dosing. Control animals received the vehicle of each drug.

2.6. Measurement of skin temperature

Skin surface temperature was measured by a handheld thermometer (HA200 K) connected to a thermocouple probe (N-211K-00-1-TC1-ASP) (Anritsu Meter Co., Tokyo, Japan) at a temperature of 22 ± 1 °C and 50% ± 10% humidity.

2.7. Statistics

The data are presented as the mean ± S.E.M. Statistical analyses were performed using Student's t-test or one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Values of P<0.05 were considered significant.

3. Results

3.1. Development of atopic dermatitis-like symptoms and prolongation of the duration of one bout of spontaneous scratching in HR-AD-fed mice

As shown in Fig. 1A, the skin of HR-AD-fed mice showed atopic dermatitis-like skin changes, including erythema, skin dryness, and wrinkling, 8 weeks after the start of feeding, whereas the skin of normal diet-fed mice exhibited no visible abnormalities. Histologically, no obvious abnormalities were seen in the skin of normal diet-fed mice (Fig. 1B), while inflammatory changes, including epidermal hyperplasia and extensive infiltration of inflammatory cells in the dermis, were observed in the skin of HR-AD-fed mice (Fig. 1C).

Table 1
Cumulative duration, frequency, and duration of one bout of scratching after intradermal administration of various doses of ethanol in normal diet- and HR-AD-fed mice 8 weeks after the start of feeding.

Solution	Concentration of ethanol (%)	Cumulative duration (s/h)		Frequency (times/h)		Duration of one bout (s)	
		Normal	HR-AD	Normal	HR-AD	Normal	HR-AD
Saline	–	8.8 ± 1.6	4.3 ± 1.3	22.0 ± 3.5	6.5 ± 2.6[b]	0.40 ± 0.03	0.84 ± 0.21[a]
Ethanol	0.01	7.4 ± 1.6	4.6 ± 3.5	18.0 ± 3.2	6.7 ± 5.0	0.42 ± 0.06	0.75 ± 0.10[a]
	0.1	6.9 ± 3.0	2.8 ± 2.4	16.8 ± 7.0	3.0 ± 2.4	0.38 ± 0.03	0.73 ± 0.13[a]
	1	4.2 ± 1.4	3.4 ± 2.4	10.7 ± 3.4[c]	4.7 ± 2.9	0.39 ± 0.03	0.70 ± 0.11[a]

Saline (20 μl/site) and ethanol solution (0.01%, 0.1%, and 1%, 20 μl/site) were intradermally and sequentially administered at 2-h intervals. Each value represents the mean ± S.E.M. of 4–6 animals. [a] and [b]: P < 0.05 and P < 0.01 vs. normal diet, and [c]: P < 0.05 vs. saline.

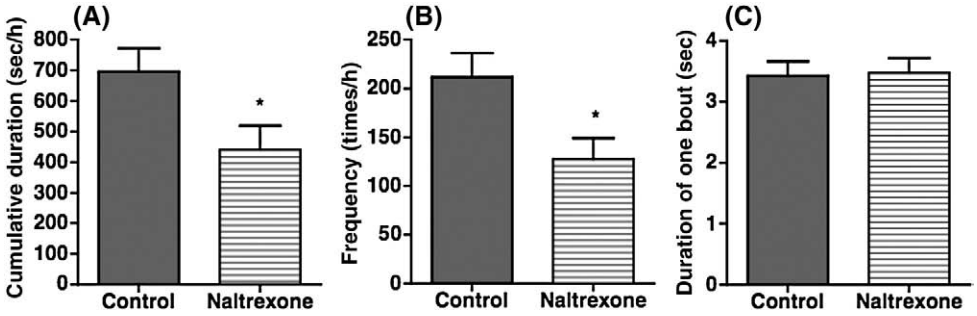

Fig. 3. Effects of naltrexone on cumulative duration (A), frequency (B), and duration of one bout (C) of ethanol-induced scratching. Naltrexone (10 mg/kg) was subcutaneously administered 30 min before ethanol administration (30%, 10 ml/kg, p.o.). Each column represents the mean ± S.E.M. of 12 or 13 animals. *$P<0.05$.

Fig. 1D–F shows the analysis of spontaneous scratching 8 weeks after the start of feeding. There was no significant difference in the cumulative duration and frequency of scratching between normal diet- and HR-AD-fed mice (Fig. 1D and E); on the other hand, the duration of one scratching bout in HR-AD-fed mice was about 2.2 times longer than that in normal diet-fed mice (Fig. 1F).

3.2. Aggravation of scratching by orally administered ethanol in HR-AD-fed mice

Fig. 2A–C shows the effects of oral administration of 10 ml/kg of 3%, 10%, and 30% ethanol solution on scratching behavior 8 weeks after the start of feeding. In normal diet-fed mice, neither the cumulative duration nor the frequency of scratching was affected by the low (3%) and the middle (10%) doses of ethanol, whereas these parameters were decreased by the high dose of ethanol (30%). In contrast, increasing doses of ethanol resulted in a slight prolongation of the duration of one scratching bout in normal diet-fed mice. On the other hand, in HR-AD-fed mice, when vehicle was administered, the frequency was less than those in normal diet-fed mice, whereas the duration of one scratching bout was significantly prolonged, similar to the result shown in Fig. 1F. Low and middle doses of ethanol did not affect any of the scratching parameters in HR-AD-fed mice. However, when the high dose of ethanol was administered to HR-AD-fed mice, all three parameters were markedly and significantly prolonged ($P<0.001$).

Fig. 2D–F shows the time-course changes in the scratching parameters after high-dose ethanol. In normal diet-fed mice, scratching episodes with duration of 0.7–1.5 s were rarely observed during the measurement period. In contrast, in HR-AD-fed mice, a large number of scratching episodes with duration of approximately 5 s were observed, with a peak 10–20 min after high-dose ethanol, followed by a gradual decline within 1 h after ethanol administration.

On the other hand, when high-dose ethanol was given orally, behavioral changes other than scratching, including ataxia and sedation/hypnosis, occurred in both groups of mice to a similar degree (data not shown). The oral administration of high-dose ethanol aggravated scratching in HR-AD-fed mice 4 weeks after the start of feeding when AD-like skin changes started to develop, though the extent of the effect was less than that at 8 weeks (data not shown).

The effects of intradermal administration of 20 μl/site of 0.01%, 0.1%, and 1% ethanol solution on scratching responses are shown in Table 1. All doses of ethanol administered intradermally failed to induce a scratching response in both normal diet- and HR-AD-fed mice.

Collectively, these results indicate that only when high-dose ethanol (30%, 10 ml/kg) was orally administered to HR-AD-fed mice, the scratching with prolonged duration of one bout was markedly increased. Thus, the effects of various drugs on the scratching after oral administration of high-dose ethanol in HR-AD-fed mice (called "ethanol-induced scratching") were examined.

3.3. Effects of various drugs on ethanol-induced scratching

Fig. 3 shows the effect of the μ-opioid receptor antagonist, naltrexone, on ethanol-induced scratching. Naltrexone at a dose of 10 mg/kg significantly inhibited both the cumulative duration and the frequency of scratching, while the duration of one bout of scratching was not affected.

The effect of application of dibucaine ointment on ethanol-induced scratching is presented in Fig. 4. Application of hydrophilic ointment

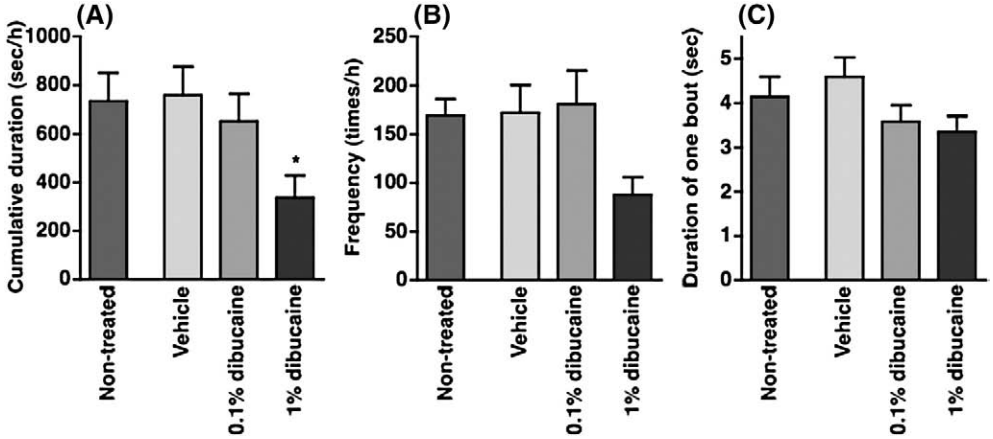

Fig. 4. Effects of dibucaine ointment on cumulative duration (A), frequency (B), and duration of one bout (C) of ethanol-induced scratching. Dibucaine ointment [0.1 and 1% (w/w)] was applied on the whole skin of mice at a weight of 150 mg/animal 1 h before ethanol administration (30%, 10 ml/kg, p.o.). Each column represents the mean ± S.E.M. of 14 or 15 animals. *$P<0.05$ vs. vehicle.

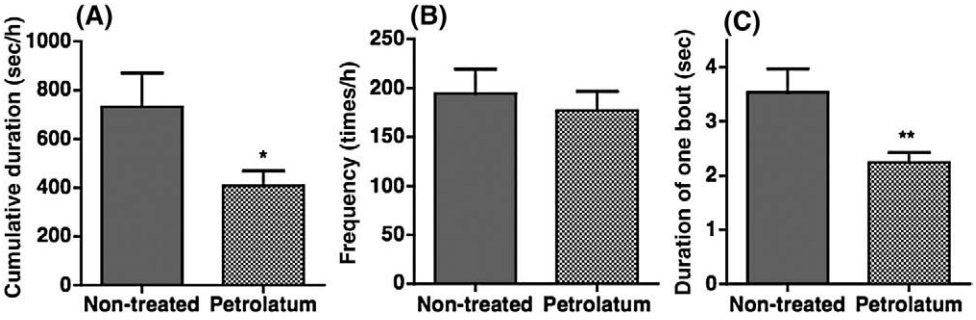

Fig. 5. Effects of petrolatum ointment on cumulative duration (A), frequency (B) and duration of one bout (C) of ethanol-induced scratching. Petrolatum ointment (200 mg/animal) was applied on the whole skin of mice immediately after ethanol administration (30%, 10 ml/kg, p.o.). Each column represents the mean ± S.E.M. of 12 animals. *P<0.05 and **P<0.01.

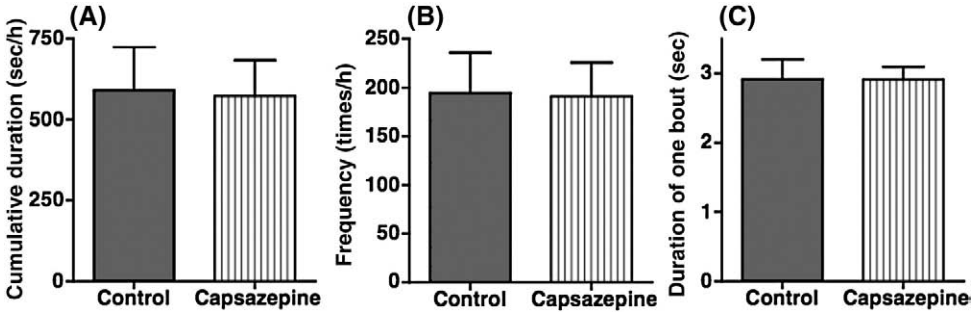

Fig. 6. Effects of capsazepine on cumulative duration (A), frequency (B), and duration of one bout (C) of ethanol-induced scratching. Capsazepine (37.7 mg/kg) was subcutaneously administered 30 min before ethanol administration (30%, 10 ml/kg, p.o.). Each column represents the mean ± S.E.M. of 12 animals.

(vehicle) had no effect on the scratching. In contrast, application of 1% dibucaine ointment attenuated ethanol-induced scratching.

Fig. 5 presents the effects of application of petrolatum ointment on ethanol-induced scratching. Petrolatum ointment was applied immediately after ethanol administration, and then scratching was analyzed for 1 h, because petrolatum ointment application transiently restores the reduced function of skin barrier only for 1 h (data not shown). Resultantly, both the cumulative duration and duration of one bout were significantly reduced by petrolatum ointment application; however, the frequency in petrolatum-treated mice was not different from that in the non-treated mice. On the other hand, ethanol administration itself did not affect the function (data not shown).

The effect of the TRPV1 antagonist, capsazepine, on ethanol-induced scratching is presented in Fig. 6. Capsazepine at a dose of 37.7 mg/kg (= 100 μmol/kg) had no effect on ethanol-induced scratching.

Fig. 7 shows the effects of the $GABA_A$ receptor antagonists, picrotoxin and bicuculline, on ethanol-induced scratching. Both antagonists produced a dose-dependent inhibition of the cumulative duration and frequency of scratching, and a significant effect was observed at 3 mg/kg. The duration of one bout of scratching was slightly shortened in picrotoxin-treated mice, while that in bicuculline-treated mice was significantly reduced at a dose of 3 mg/kg.

The effect of intracisternal administration of NMDA on ethanol-induced scratching is shown in Fig. 8. The cumulative duration and

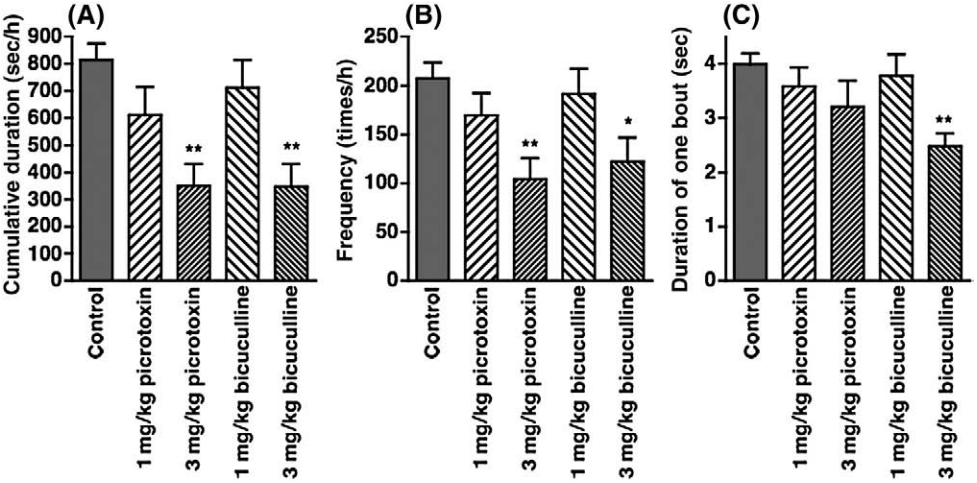

Fig. 7. Effects of picrotoxin and bicuculline on cumulative duration (A), frequency (B), and duration of one bout (C) of ethanol-induced scratching. Picrotoxin (1 and 3 mg/kg) or bicuculline (1 and 3 mg/kg) was intraperitoneally administered immediately after ethanol administration (30%, 10 ml/kg, p.o.). Each column represents the mean ± S.E.M. of 12 animals. *P<0.05 and **P<0.01.

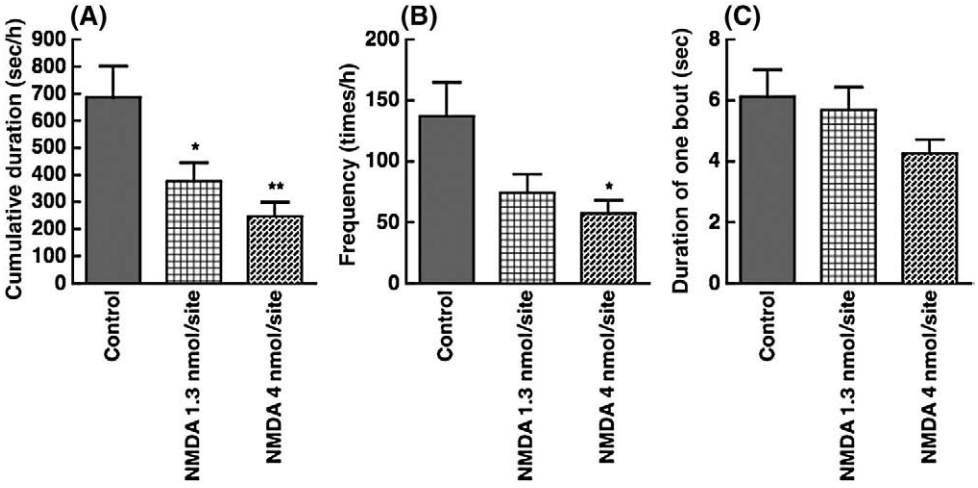

Fig. 8. Effects of NMDA on cumulative duration (A), frequency (B), and duration of one bout (C) of ethanol-induced scratching. NMDA (1.3 and 4 nmol/10 μl/site) was intracisternally administered 10 min after ethanol administration (30%, 10 mg/kg, p.o.). Each column represents the mean ± S.E.M. of 14 or 15 animals. *$P<0.05$ and **$P<0.01$.

frequency of scratching were significantly and dose-dependently inhibited by the intracisternally administered NMDA. The duration of one bout of scratching tended to be decreased in NMDA-treated mice.

3.4. Skin temperature changes after ethanol administration

Fig. 9 presents the skin temperature changes that occurred during the oral administration of high-dose ethanol (30%, 10 ml/kg). Before the ethanol was given, the skin temperature of HR-AD-fed mice was lower than that of normal diet-fed mice. The oral administration of the low (3%, 10 ml/kg) and middle (10%, 10 ml/kg) doses of ethanol had no significant effect on skin temperature (data not shown), while the skin temperature significantly declined in both groups of mice given high-dose ethanol.

4. Discussion

We have analyzed scratching in mice based on three behavioral parameters (cumulative duration, frequency, and the duration of one bout of scratching). The cumulative duration is dependent on both the frequency and the duration of one bout of scratching. As described in our previous reports (Fujii et al., 2005, 2006) and in the present study (Fig. 1), although the frequency of spontaneous scratching in HR-AD-fed mice was not constantly changed, the duration of one bout of scratching was reproducibly prolonged. This implies that scratching with a long duration appears variably in HR-AD-fed mice, but rarely in normal diet-fed mice. A similar finding has been reported in the NC/Nga mouse, which is a well-known model for atopic dermatitis; long duration scratching (more than 1 s) was increased, while short duration scratching (less than 1 s) was frequently observed independently of the development of atopic dermatitis-like symptoms (Takano et al., 2003). In patients with atopic dermatitis, an increased total scratching time during sleep has been shown to be dependent on a longer duration of one bout of scratching rather than a higher frequency of scratching (Ebata et al., 1999). Thus, scratching with a long duration in HR-AD-fed mice could represent some of the itching sensation in atopic dermatitis. On the other hand, intradermal injection of pruritogens in intact mice has been shown to increase the frequency of scratching (Inagaki et al., 2003; Nojima and Carstens, 2003). Therefore, although it is difficult to clearly define the difference between the frequency and the duration of one bout of scratching, both parameters appear to reflect the itching sensation.

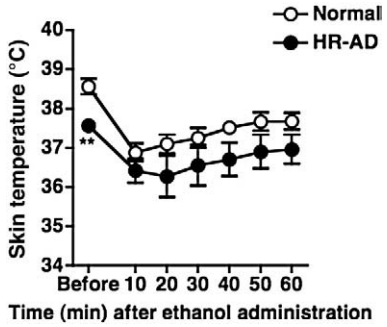

Fig. 9. Skin temperature changes after ethanol administration (30%, 10 mg/kg, p.o.) in normal diet- and HR-AD-fed mice. Each point represents the mean ± S.E.M. of 6 animals. **$P<0.01$.

The most interesting finding in the present study was that orally administered ethanol markedly aggravated scratching in HR-AD-fed mice developing atopic dermatitis, but not in normal diet-fed mice with intact skin (Fig. 2). Additionally, the degree of aggravation of scratching correlated with the degree of atopic dermatitis-like symptoms (data not shown). These results thus suggest that the aggravation of scratching is closely linked to the development of atopic dermatitis-like symptoms. However, since the dose of ethanol needed to aggravate scratching in the mice (30% ethanol, 10 ml/kg) is considered to be very high, this aggravation may not reflect the clinical characteristics in atopic dermatitis patients. Nevertheless, the aggravated scratching was suppressed by naltrexone (Fig. 3) and dibucaine (Fig. 4), which attenuate various itching sensations in humans (Bernstein et al., 1982; Shuttleworth et al., 1988; Metze et al., 1999), suggesting that the scratching response shares some common mechanisms with human itching.

Previous studies have suggested that skin barrier dysfunction is involved in the basal scratching in HR-AD-fed mice (Fujii et al., 2006). Thus, we examined whether ethanol-induced scratching is also related to the skin barrier dysfunction. Resultantly, ethanol-induced scratching was suppressed by improvement of skin barrier after an application of petrolatum ointment (Fig. 5), while ethanol administration itself did not affect the function of skin barrier (data not shown). These results thus suggest that ethanol indirectly aggravates the basal scratching.

Next, the actions of ethanol involved in the aggravation of scratching were investigated. A recent study has shown that ethanol directly stimulates the primary afferent nerves via TRPV1, followed by substance P release and local neurogenic inflammation in guinea pig airways (Trevisani et al., 2004). It has also been reported that an intradermal injection of substance P elicits itch-related scratching at the injected site in mice (Andoh et al., 1998). These studies prompted

us to examine the effects of the TRPV1 antagonist, capsazepine, on ethanol-induced scratching. However, unexpectedly, capsazepine did not affect the scratching (Fig. 6), suggesting no involvement of TRPV1 in the aggravation of scratching. Furthermore, intradermal (Table 1) or topical (Fujii et al., unpublished data) application of ethanol did not induce any scratching response. Collectively, these results imply that the aggravation is not due to direct actions of ethanol on the peripheral site.

There has been an accumulation of evidence that ethanol exerts its depressant effects on the CNS by binding with and altering the function of some ligand-gated ion channels (Harris, 1999). Of the channels involved, $GABA_A$ receptors are potentiated by ethanol via facilitating the opening of channels (Grobin et al., 1998). Thus, the effects of $GABA_A$ receptor antagonists, picrotoxin and bicuculline, on ethanol-induced scratching were examined; both antagonists significantly suppressed the scratching response (Fig. 7). Furthermore, our preliminary experiment revealed that i.p. administration of pentobarbital, which potentiates $GABA_A$ receptor function as well as ethanol, also resulted in aggravation of scratching with a long duration in HR-AD-fed mice (Fujii et al., unpublished data). Therefore, these results strongly support an important role for $GABA_A$ receptor-mediated action in ethanol-induced scratching. On the other hand, we recently found that i.p. administration of the $GABA_A$ receptor selective agonist, mucimol, did not elicit any scratching response in HR-AD-fed mice, even when CNS depressant effects were observed (Fujii et al., unpublished data). This discrepancy between the results of the antagonists and the agonist may be due to the fact that ethanol acts on several CNS receptors other than $GABA_A$ receptors, including NMDA receptors (Hoffman et al., 1989), glycine receptors (Valenzuela et al., 1998), nicotinic ACh receptors (Cardoso et al., 1999), and serotonin 5-HT_3 receptors (Lovinger, 1999). Furthermore, the function of NMDA receptors has been shown to be inhibited by ethanol (Kumari and Ticku, 2000). Thus, we tested the effect of NMDA receptor activation on the CNS by intracisternal administration of NMDA and observed its effect on ethanol-induced scratching; NMDA administration significantly inhibited the scratching response (Fig. 8). Therefore, this finding leads to the hypothesis that ethanol aggravates the scratching by simultaneously and probably synergistically altering the functioning of CNS proteins, such as $GABA_A$ receptors and NMDA receptors, though further investigations are warranted to clarify the precise mechanisms.

Clinically, the itching sensation can be modulated by mental distraction. Distraction has also been found to inhibit scratching behavior in mice (Yamaguchi et al., 2001). These observations imply that there must be a neural pathway within the CNS that plays a role in modulating itching. Furthermore, a recent positron emission tomography study of itch inhibition by painful cold stimuli supported the presence of an itch modulation system in the human brain; activation of periaqueductal gray matter, which is known to be one of the central pain modulation systems, might be involved in the central inhibition of itching (Mochizuki et al., 2003). On the other hand, Bovier et al. (1984) reported that ethanol increased the escape latency and the threshold for aversive electrical stimulation of the periaqueductal gray matter. This suggests that ethanol interferes with the descending pathway from the periaqueductal gray matter. Thus, these findings raise the possibility that ethanol-induced scratching might be caused by suppression of the central itch modulation system.

Ethanol intake is known to cause a feeling of warmth, because ethanol increases cutaneous blood flow by modulation of central vasomotor control mechanisms (Malpas et al., 1990). In addition, it has been shown that warming the skin aggravates itching, while cooling the skin suppresses it (Mizumura and Koda, 1999). Thus, tissue (skin) temperature change may be involved in ethanol-induced scratching. However, the present data indicated that the skin temperature in HR-AD-fed mice was lower than that in normal diet-fed mice, and that the high dose of ethanol produced a significant fall in skin temperature, both in normal diet- and HR-AD-fed mice (Fig. 9). From this, it appears that skin temperature change is not greatly involved in the scratching response.

In summary, orally administered ethanol markedly aggravated itch-related scratching with a long duration in HR-AD-fed mice developing atopic dermatitis. Furthermore, the CNS depressant effects of ethanol appear to play a major role in the aggravation of scratching. On the other hand, it is known that, in patients with atopic dermatitis, nocturnal aggravation of pruritus is prominent (Yosipovitch et al., 2002). Since ethanol, as well as barbiturates, depresses CNS functioning, progressively producing sleep (pharmacological hypnosis), the ethanol-induced scratching in HR-AD-fed mice may mimic the nocturnal scratching in human atopic dermatitis. Studies are in progress to further characterize the itch-related scratching responses in this mouse model.

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