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Chronic psychological stress exaggerates the compound 48/80-induced scratching behavior of mice

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ABSTRACT

Although accumulating clinical evidence has shown that psychological stress worsens cutaneous symptoms by exaggerating scratching behavior, how the stress affects the scratching is unclear. Therefore, we herein investigated this using an animal model of scratching. Male BALB/c mice were exposed to 1 h water avoidance stress (WAS) for ten consecutive days. Twenty-four hours after the last stress session, the mice were injected into the back of the neck with a condensation product of *N*-methyl-*p*- methoxyphenethylamine with formaldehyde (compound 48/80), and their scratching behavior was then observed for 120 min. Mast cell number in the skin and histamine and corticosterone levels in the plasma were examined. The scratching number was significantly higher in the chronic WAS group than in the control group. Both mast cell number in the skin and the peak histamine in the plasma after the compound 48/80 injection were also significantly higher in the chronic WAS group in comparison to the control group. Chronic WAS delayed the peak corticosterone plasma response to the compound 48/40 injection. These findings indicate that chronic WAS exacerbates the compound 48/80-induced scratching behavior of mice. Both the increased number of skin mast cells and delayed glucocorticoid reaction may be related to this exacerbation.

1. Introduction

In pruritic cutaneous diseases such as atopic dermatitis (AD) and urticaria, it is clinically important to control the itching sensation, because this uncomfortable sensation often elicits scratching behavior, which consequently induces an exacerbation of the skin condition (Ebata et al., 1999). For example, in patients with AD, nocturnal total scratching time is correlated with the severity of the disease (Wahlgren, 1999). It was also reported that AD patients show "butterfly sign" which refers to a butterfly-shaped area of relatively decreased pigmentation over the upper part of the back where they are unable to reach to scratch (Kimura and Miyazawa, 1989). These findings suggest that the scratching behavior is not just a reflection of itching sensation, but a determinant factor of exacerbating skin condition; therefore, controlling the scratching behavior is very important in the treatment of patients with itchy cutaneous skin diseases.

Since several decades ago, a number of studies have shown the relationship between the exacerbation of dermatitis and environmental factors including the emotional stress of AD patients (Arck et al., 2006; Greenhill and Finesinger, 1942; King and Wilson, 1991; Kodama et al., 1999; Wittkower and Edgell, 1951). Such relationship between stress and dermatitis has also been studied in AD animal models. For example, Orita et al. (2010) showed that strong exercise stress exacerbates dermatitis, while proper exercise reduces it in NC/Nga mice, an AD animal model sensitized to house dust mites. Interestingly, Kobayashi (2000) reported that "habitual scratching behavior", a different type of scratching from an ordinary scratching caused by skin itching, is often induced by emotional stress in AD patients. These findings, taken together, suggest the possible role of stress in the aggravation of the cutaneous lesions via a stress-itch-scratch vicious cycle; therefore, it is important to clarify how and to what extent emotional stress affects the strength of scratching behavior.

Kuraishi et al. (1995) reported an animal model for evaluating the strength of itching. In this report, they showed that the subcutaneous injection of pruritogenic agents, such as a condensation product of *N*-methyl-*p*-methoxyphenethylamine with formaldehyde, compound 48/80, elicit scratching of the treated skin in a dose dependent manner, while algesiogenic agents, such as capsaicin and formalin, fail to show any apparent behavioral effect. This experimental system using compound 48/80 has since been verified by several other researchers and is now established as a standard method for evaluating the effect of anti-allergic drugs (Patel and Dong, 2010).

In the current study, in order to evaluate the impact of stress on the extent of scratching, we examined the effects of water avoidance stress (WAS) (Ibeakanma et al., 2011), a frequently used paradigm of psychological stress, on scratching behavior induced by compound 48/80.

Abbreviations: AD, atopic dermatitis; compound 48/80, a condensation product of *N*-methyl-*p*-methoxyphenethylamine with formaldehyde; HPA, hypothalamic–pituitary–adrenal; WAS, water avoidance stress.

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2. Materials and methods

2.1. Animals

Male BALB/c mice (7–8 weeks of age) were obtained from Charles River Japan (Shizuoka, Japan) and were individually maintained at a constant temperature (23–25 °C) on a 12-h light/12-h dark cycle with food and water freely available. Experiments were always done in the morning (between 9:00 a.m. and 12:00 p.m.) to minimize variation due to circadian rhythmicity. All experiments were approved by the Ethics Committee on Animal Experiments of the Graduate School of Medical Sciences, Kyushu University.

2.2. Stress protocol

Exposure to WAS was performed as described previously (Hong et al., 2009). Briefly, mice were placed on a glass platform (diameter, 7 cm) in the middle of a plastic container filled with water to 1 cm below the height of the platform. The mice were subjected to WAS for 1 h per day in the morning for ten consecutive days. This well-characterized WAS represents a potent psychological stressor with large elevations of ACTH and corticosterone within 30 min (Million et al., 1999). Control animals without any stress were isolated in an individual cage for the same duration as the mice undergoing stress exposure.

2.3. Experimental protocol

The observations of scratching behavior were carried out according to the previous method with some modifications (Hirayama et al., 2003). Twenty-four hours after the last WAS session, a total of 10 μg of compound 48/80 (Sigma, St. Louis, MO) was dissolved in 50 μl saline and injected subcutaneously into the back of the neck. Immediately after the injection, each mouse was put back into an individual cage and the scratching behavior was then recorded for 120 min using an 8-mm video camera (CCD-TRV86, Sony, Tokyo, Japan) under quiet conditions. In general, the mice showed several scratches of the injected site for about 1 s and such scratching behavior was counted as one bout of scratching. Every bout of scratching was counted across each 10 min bin. In analyzing the experimental data, any information as to which group was exposed to WAS or treated with drugs was blinded to the observer.

2.4. Measurement of plasma histamine and corticosterone levels

To examine time-course changes in plasma histamine and corticosterone levels, the mice were sacrificed by a cervical dislocation before, and 15, 30, 60 and 120 min after a subcutaneous injection of compound 48/80. Whole blood was obtained by a cardiac puncture using a heparinized syringe, and then was collected in EDTA-coated sample tubes. The plasma obtained by centrifugation was stored at −80 °C for histamine and corticosterone determination later. The plasma histamine and corticosterone levels were measured using commercially available ELISA kits (histamine, SPI-BIO, Paris, France; corticosterone, Cayman Chemical Company, Ann Arbor, MI).

2.5. Histologic examination

The formalin-fixed sections of skin tissue from the back of the neck were collected from the WAS mice at 24 h after the last WAS stress session and from the control mice without WAS. The number of skin mast cells per low power field (×100) was counted using toluidine blue staining, as previously described (Singh et al., 1999). All mast cells were counted by two different researchers, blinded to the experimental conditions.

2.6. Statistical analysis

All data are expressed as mean ± SE. Comparison of the number of skin mast cells of the groups was analyzed using the unpaired *t*-test. Statistical differences between the groups were determined by a repeated measures analysis of variance followed by the unpaired *t*-test. A value of $P < 0.05$ was considered to be significantly different from the corresponding value.

3. Results

3.1. Chronic exposure to WAS exaggerates scratching behavior

To clarify whether or not psychological stress affects the scratching behavior, the effect of chronic WAS stress on the number of scratching was evaluated in the mice given a subcutaneous injection of compound 48/80.

As shown in Fig. 1, chronic WAS induced a significant increase in the number of scratchings ($F(1, 12) = 1.49$, $P < 0.01$). Interestingly, the enhanced effects of chronic WAS on the scratching number were evident during the early-phase (≤60 min) of the observation period examined.

3.2. Effects of chronic WAS on the number of skin mast cells and the compound 48/80-induced plasma histamine levels

As shown in Fig. 2, the number of skin mast cells was significantly higher in the chronic WAS group than in the control group (control 20.2 ± 1.9 cells per low power field, WAS 27.0 ± 1.3* cells per low power field, $^{*}P < 0.05$). Moreover, the plasma histamine level at 15 min after the compound 48/80 injection was also significantly higher in the chronic WAS group than in the control group (Fig. 3, control 87.8 ± 5.5 ng/ml, WAS 135.0 ± 20.1* ng/ml, $^{*}P < 0.05$).

3.3. Chronic WAS delays the peak corticosteroid response to subcutaneous compound 48/80 injection

Our previous report showed that endogenous glucocorticoids play an important role in the suppression of the compound 48/80-induced scratching; hence, our final experiment investigated the kinetics of plasma corticosterone levels after the administration of compound 48/80.

As shown in Fig. 4, the peak corticosterone elevation in plasma was seen at 15 min after the compound 48/80 injection in the control group, while it was at 30 min after the injection in the WAS group (15 min, control 407.5 ± 86.7** ng/ml, WAS 63.0 ± 11.0 ng/ml; 30 min, control 163.9 ± 31.0* ng/ml, WAS 488.2 ± 106.9 ng/ml, $^{*}P < 0.05$, $^{**}P < 0.01$).

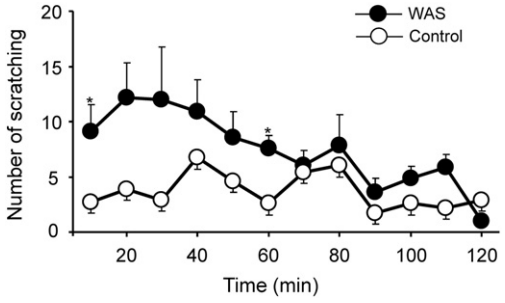

Fig. 1. Effects of chronic WAS on compound 48/80-induced scratching behavior. Mice exposed to chronic WAS were given an injection of compound 48/80, and their scratching behavior was evaluated, as described in the Materials and methods section. All data are expressed as the mean ± S.E. (n = 7 for each group). $^{**}P < 0.01$ and $^{*}P < 0.05$ were considered to be significantly different from the corresponding values in the control group.