



N-Acetyl- β -endorphin suppresses atopic dermatitis in NC/Nga mice

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Abstract

Background: Atopic dermatitis (AD) is known to be affected by neuropeptides. However, the mechanism underlying this phenomenon is unclear. This study analyzed the mechanism(s) responsible for the influence of β -endorphin (β -End) or N-acetyl- β -endorphin (acetyl- β -End) on AD.

Methods: Specific pathogen-free (SPF) and conventional NC/Nga mice were used for the studies. Conventional (not SPF) mice, spontaneously develop dermal symptoms similar to that of patients with AD. In the present study we treated mice with 5 μ g/mouse of β -End or acetyl- β -End for 20 days. In addition, a histone acetyltransferase inhibitor II (HAT inhibitor, 100 mg/kg) was given for 20 days to examine its effects on the acetylation of the β -End.

Results: The symptoms of the conventional group were ameliorated by both β -End and acetyl- β -End treatment, although acetyl- β -End treatment more effectively relieved the symptoms than β -End, and the improvement induced by the β -End treatment disappeared following HAT inhibitor treatment.

Conclusions: These observations suggested that β -End or acetyl- β -End treatment can suppress the symptoms of AD in the mice, and that the effect of the β -End is induced by the acetylation of the β -End by HAT.

Keywords: Atopic dermatitis, β -Endorphin, acetyl- β -Endorphin, histone acetyltransferase inhibitor II

Introduction

Various hormones are secreted after exposure to stress, including adrenocorticotrophic hormone, cortisol, and adrenaline. The secretion of β -endorphin (β -End) is also induced by stress in animals [1,2] and humans [3,4]. β -End is a 31-amino-acid opioid synthesized in the arcuate nucleus that inhibits several central nervous system functions. In the periphery, endorphins are synthesized in the intermediate pituitary or its vestigial region. Immune cells also synthesized β -End [5], and the regulation of the immune system by β -End has been reported. Natural cytotoxicity (NK cell efficacy) was stimulated by β -End [6]. In patients with organ-specific autoimmune disease, the β -End concentration is decreased and the cytokine pattern of T-helper cells is shifted to the Th1-type [5]. Furthermore, it has been suggested that there are relationships among AD, β -End and allergies [7,8]. In our previous study using NC/Nga mice, we showed that AD-like symptoms were exacerbated by strong stress and reduced by mild stress [9].

NC/Nga mice were established as an inbred strain from Japanese fancy mice in 1957, and have recently been shown to spontaneously develop AD-like dermatitis with immunoglobulin E hyperproduction under air-uncontrolled, conventional circumstances [10,11]. The plasma level of β -End was found to be increased in mice with a mild stress load [9]. This suggested the possibility that β -End contributed to the reduction of the AD-like symptoms.

In this study, to examine the mechanism underlying the AD-like symptom improvement after mild stress and the potential involvement of β -End in this effect, we examined the effects of β -End and acetyl- β -End in NC/Nga mice with AD.

Materials and methods

Animals

Conventional and specific pathogen-free (SPF) NC/Nga male mice (7 weeks old) were purchased from SLC (Hamamatsu, Aichi, Japan). They were housed in rooms with a 12-h light/12-h dark cycle, and all animals were allowed free access to laboratory chow (CE-2, Oriental Yeast Co., Tokyo, Japan) and water during the experiments. These animals were subjected to experiments according to the animal care regulations of Osaka City University Medical School. The mice were divided into five groups ($n=10$) and used at same time. In addition, each experiment was repeated three times.

Evaluation of the inflammatory score

The symptoms of dermatitis in these animals were evaluated on day 9 in the rostral skin, and the severity of the edema, erythema and hemorrhage was scored (0, none; 1, slight; 2, moderate; 3, severe) as described previously [10].

Treatment of animals with β -End and acetyl- β -End

Approximately 5 μ g/mouse of β -End or acetyl β -End in 50 μ l

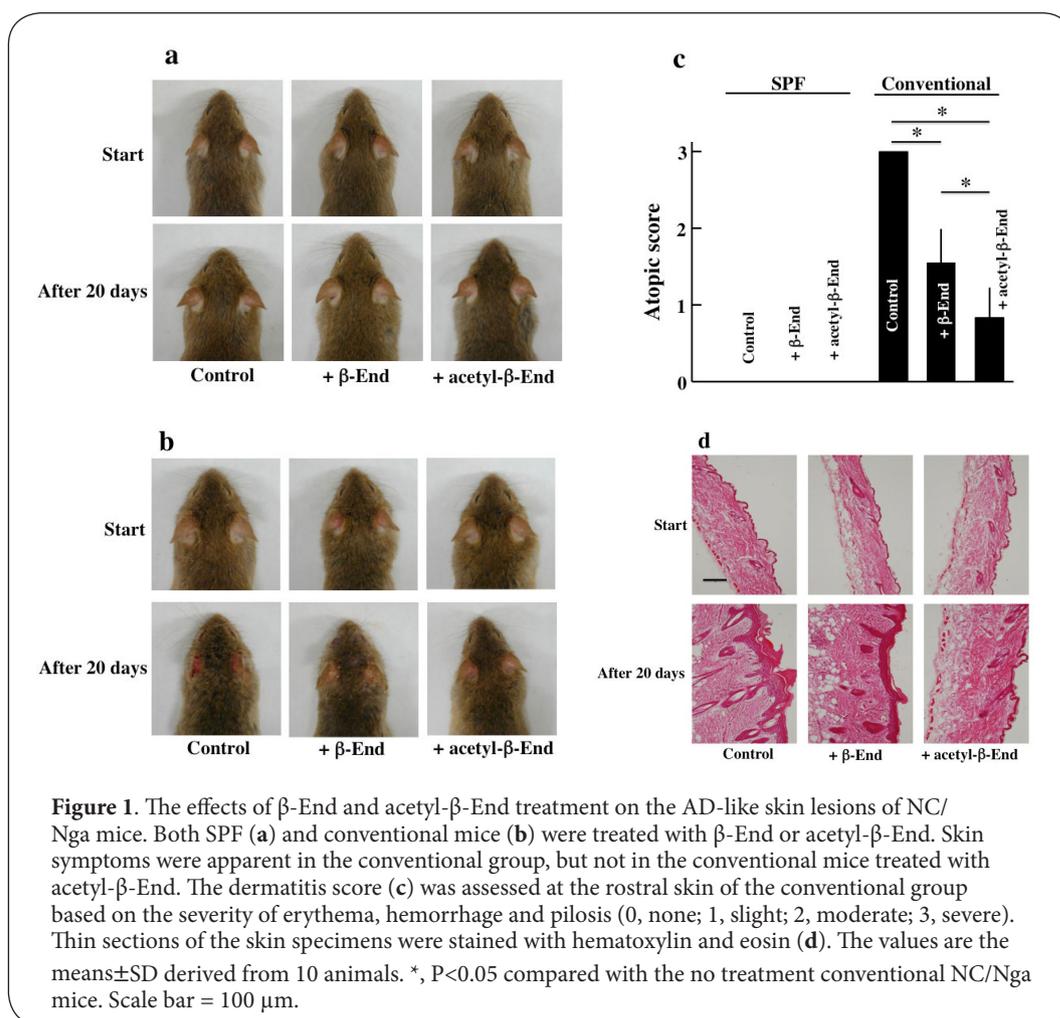


Figure 1. The effects of β -End and acetyl- β -End treatment on the AD-like skin lesions of NC/Nga mice. Both SPF (a) and conventional mice (b) were treated with β -End or acetyl- β -End. Skin symptoms were apparent in the conventional group, but not in the conventional mice treated with acetyl- β -End. The dermatitis score (c) was assessed at the rostral skin of the conventional group based on the severity of erythema, hemorrhage and pilosis (0, none; 1, slight; 2, moderate; 3, severe). Thin sections of the skin specimens were stained with hematoxylin and eosin (d). The values are the means \pm SD derived from 10 animals. *, $P < 0.05$ compared with the no treatment conventional NC/Nga mice. Scale bar = 100 μ m.

saline was intraperitoneally injected [11] throughout the experimental period (the injection once a day for 20 days), whereas saline was injected into control mice.

HAT inhibitor treatment

Approximately 100 mg/kg of a histone acetyltransferase inhibitor II (HAT inhibitor, Merck, Darmstadt, Germany) in 1% DMSO [12] was injected intraperitoneally into the mice throughout the experimental period (injection every other day for 20 days), while 1% DMSO was injected into the control mice. In addition, as a judgment result of the atopic score of the dorsal skin by the dosises 1, 5 and 10 μ g/mouse of acetyl- β -End, we used the smallest dose (5 μ g/mouse) that showed the significant amelioration effect (Data not shown).

Preparation and staining of dorsal skin

For the histological studies, the mice were killed on the final day of the examination. The dorsal skin specimens were fixed in phosphate-buffered paraformaldehyde (4%), embedded in frozen Tissue Tek, OCT compound, and cut into 5 μ m thick

sections. These thin sections were stained with HE according to the standard procedure.

Analysis of IgE and β -End in plasma

Under light ether anesthesia, blood samples were obtained by cardiac puncture the final day of the examination. After centrifugation, plasma samples were obtained and analyzed for IgE and β -End. Plasma levels of IgE and β -End were determined by using ELISA kits according to manufactures instructions. ELISA kits for IgE and β -End were obtained from Yamasa Shoyu Co. (Chiba, Japan) and Phoenix Pharmaceuticals (CA, USA), respectively.

Statistical analysis

All data are presented as the means \pm SD derived from 10 animals. The results obtained from two animal groups were analyzed by either Student's *t*-test or an ANOVA using a computer software package. Data were compared using an ANOVA with the use of the Stat-View 512 software program. Differences were considered to be significant for values of $p < 0.05$.

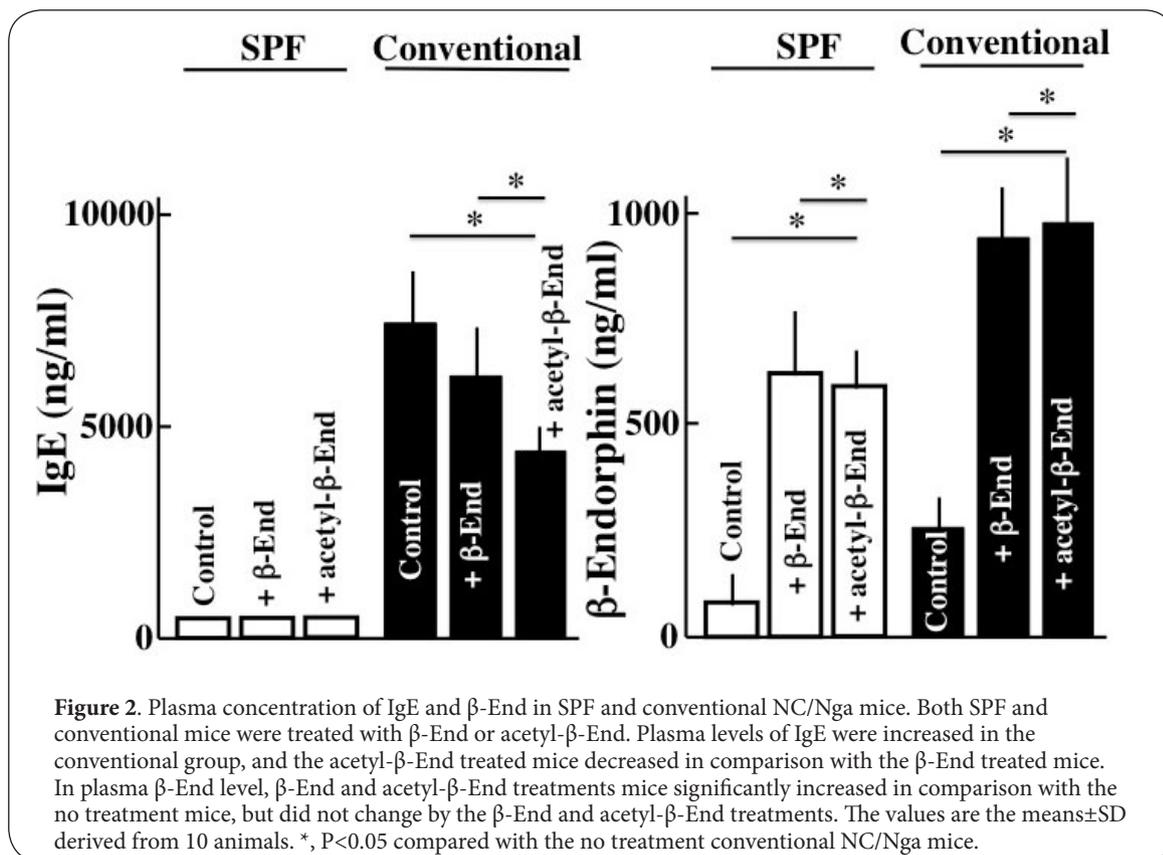


Figure 2. Plasma concentration of IgE and β -End in SPF and conventional NC/Nga mice. Both SPF and conventional mice were treated with β -End or acetyl- β -End. Plasma levels of IgE were increased in the conventional group, and the acetyl- β -End treated mice decreased in comparison with the β -End treated mice. In plasma β -End level, β -End and acetyl- β -End treatments mice significantly increased in comparison with the no treatment mice, but did not change by the β -End and acetyl- β -End treatments. The values are the means \pm SD derived from 10 animals. *, $P < 0.05$ compared with the no treatment conventional NC/Nga mice.

Results

Effect of β -End or acetyl- β -End treatment on AD mice symptoms

Although the SPF group did not exhibit symptoms of AD under any of the conditions tested (Figure 1a,1c and 1d), the conventional mice started to exhibit symptoms including edema, erythema and hemorrhage on their rostral skin (Figure 1b,1c and 1d). The dermal symptoms were ameliorated in the groups treated with β -End or acetyl- β -End. In addition, the acetyl- β -End-treated mice showed more improved skin symptoms compared to the β -End treated mice.

Effect of exercise on plasma levels of cytokines and IgE

As shown in Figure 2, plasma levels of β -End in the SPF animal group significantly increased by treatments of β -End or acetyl- β -End. Under identical conditions, plasma levels of IgE remained low levels in SPF group. The plasma levels of β -End and IgE were significantly high as compared with those in SPF group particularly when the symptom of dermatitis became apparent. Plasma levels of β -End were markedly elevated in the conventional group, no appreciable effect of β -End and acetyl- β -End treatments were observed. Plasma levels of IgE were markedly elevated in the conventional groups. Although plasma levels of IgE in the conventional group were

significantly suppressed by receiving acetyl- β -End.

HAT inhibitor treatment diminishes the response to β -End treatment

Conventional mice exhibited symptoms characteristic of AD, including edema, erythema and hemorrhage of their rostral skin. The dermal symptoms were ameliorated in the animals treated with β -End (Figure 3a-3c). In contrast, the symptoms were unaffected in the animals that had been treated with both β -End and the HAT-inhibitor. In addition, there was not the effect in HAT-inhibitor alone.

Discussion

The present study demonstrated that the AD-like symptoms of the conventional mice were successfully suppressed by treatment with β -End or acetyl- β -End. Furthermore, the acetyl- β -End treatment provided better symptom relief compared to the β -End treatment. In addition, HAT treatment abolished the AD-like skin symptom reduction provided by the β -End treatment.

The plasma β -End level serves as an independent and important factor influencing the development of pruritus in AD. Opioid peptides and their G-specific receptors modulate the function of calcium channels specifically on unmyelinated

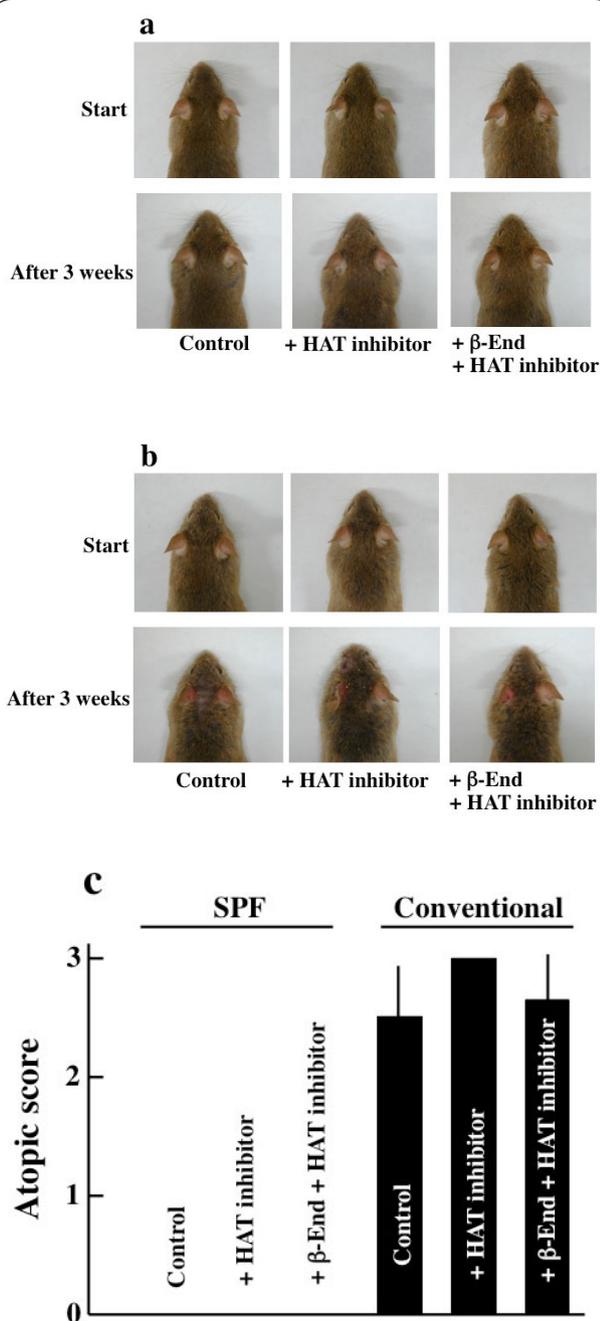


Figure 3. The effects of HAT inhibitor treatment on the AD-like skin lesion of NC/Nga mice. Both SPF (a) and conventional (b) mice were exposed to either HAT inhibitor or β -End + HAT inhibitor treatment. In the mice treated with β -End alone, the skin symptoms were reduced compared to the conventional mice with no treatment (Figure 1b), but the symptoms were increased in the mice with HAT inhibitor or β -End + HAT inhibitor treatment. The dermatitis score (c) was assessed at the rostral skin of the conventional group based on the severity of erythema, hemorrhage and pilosis (0, none; 1, slight; 2, moderate; 3, severe). The values are the means \pm SD derived from 10 animals.

c-fibers of the central nervous system, and thus, are thought to be involved in the central itch-regulatory mechanism [13]. Some reports suggest that endogenous opioid peptides may be involved as central mediators of itching [14,15]. β -End, which belongs to the endogenous opiate family, is generated upon stimulation of the pituitary-adrenal axis after stress [16,17]. The present study also demonstrated that the administration of exogenous β -End decreased the symptoms of AD and increased the expression of the μ -opioid receptor (data not shown). These results suggested that the plasma levels of β -End and the expression of the μ -opioid receptor affect the symptoms of AD.

Previous studies [18,19,20] reported that acetyl- β -End, which binds with very low affinity to opioid receptors [21] and lacks antinociceptive activity, exhibited a differential modulatory effect on the supraspinal antinociception mediated via the μ -opioid and α -2 adrenergic, but not δ -opioid, receptors in mice. It therefore seemed possible that acetyl- β -End exerted this modulation by functionally acting on a substrate coupled to both types of receptors, presumably the G proteins. In addition, Sanchez-Blazquez and Garzon [2] reported that the concentration of the opioid antagonist controls the nerve excitement induced by substance P (SP), causing the itch. Of note, the acetyl- β -End treatment more strongly controls the reaction of the SP.

The HAT inhibitor used by the examination makes H3 region of the histone the main targets. Since H3 was important for an acetylation as a core histone, we used this inhibitor. If other inhibitor is used, it will be thought that the elucidation of a deeper mechanism is possible. Therefore, the further research using other HAT inhibitor is needed.

Conclusions

These results suggest a modulatory role for the SP system and the neuropeptide acetyl- β -End on μ - and α -2 receptors in NC/Nga mice.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	KH	HY	KI
Research concept and design	√	√	√
Collection and/or assembly of data	--	--	--
Data analysis and interpretation	√	√	--
Writing the article	√	√	--
Critical revision of the article	√	--	--
Final approval of article	√	--	--
Statistical analysis	--	--	--
Contributed essential reagents and tools	--	--	√

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