Effects of Naltrexone on Spontaneous Itch-Associated Responses in NC Mice With Chronic Dermatitis

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ABSTRACT—The effects of the opioid antagonist naltrexone on spontaneous itch-associated behaviors and cutaneous nerve activities were examined to determine whether it inhibit pruritus through peripheral action in NC mice with chronic dermatitis. Their rostral-back scratching and caudal-back biting were 19 and 3.4 times more, respectively, than those of control mice. The activities of cutaneous nerves innervating the rostral and caudal back were 9.5 and 5.4 times more, respectively, in affected mice than in control mice. Subcutaneous injections of naltrexone significantly inhibited the scratching and biting, without effects on the nerve activities. The results suggest that the peripheral action does not play a central role in inhibiting chronic itch-associated behaviors.

Keywords: Itch-associated behavior, Naltrexone, NC mice

Itch is the common symptom of dermatitis and provokes the scratching of the affected skin, which makes the dermatitis worse, but its pathophysiological mechanisms remain poorly understood. There are some reports that naltrexone and the other opioid antagonists relieve itch of patients with pruritic disease such as atopic dermatitis (1), uremic pruritus (2) and chronic cholestasis (3), but the site of antipruritic action of opioid antagonists is unclear.

NC mice are useful as a mouse model for atopic dermatitis, because they show atopic dermatitis-like skin lesions and elevated serum concentration of immunoglobulin E (4 – 6). When kept long (more than 4 weeks) under the conventional environment, dermatitis and spontaneous scratching of the face, ears and the rostral part of the body are developed; there are no apparent sex differences in pathological conditions (5, 6). Spontaneous scratching may be primarily due to cutaneous pathology, but changes in central gene expression have been also implicated (5, 6). The opioid antagonist naltrexone inhibits the spontaneous scratching (6), but the site of action remains unclear. Therefore, to determine whether opioid antagonist inhibits pruritus through peripheral action, we examined the effects of naltrexone on itch-associated behaviors and cutaneous nerve activities. Opioid peptides are synthesized and processed within various types of immune cells at the site of inflammation (7). Thus, we examined the effects on itch-associated responses of the skin with and without dermatitis (rostral and caudal back, respectively); scratching of the rostral back (8) and biting of the caudal back (9) were used as an index of itching.

Twenty-eight NC mice of either sex (30 – 55-week-old) were used. They were bred in a specific pathogen-free (SPF) environment at Animal Laboratory Center, Toyama Medical and Pharmaceutical University. Experiments were conducted with permission of the Committee for Animal Experiments in Toyama Medical and Pharmaceutical University. Ten mice were kept under an SPF environment throughout the experiment (designated as SPF mouse); they didn’t have any apparent dermatitis during the experimental period. Eighteen mice were transferred to the conventional environment at 4 weeks of age (designated as CNV mouse). To increase the incidence of dermatitis and scratching, they were kept together with mice with chronic dermatitis. All CNV mice used had wispy fur on the face and rostral back and bleeding on the ears, whereas the caudal back had outwardly fine fur and no apparent lesions. Regardless of the keeping environment (SPF or conventional), the animals were housed under controlled temperature (23 – 25°C) and light (lights on from 08:00 to 20:00). Food and water were freely available.

The mouse was individually put into a clear plastic cage and after the 60-min acclimation period, the behaviors were videotaped for 60 min. The playback served for the obser-
vation of scratching and biting behaviors; a series of scratching and biting behaviors were counted as one bout of event. The activity of cutaneous nerve was recorded, as described (10). Briefly, the day after behavioral experiment, the mouse was anesthetized with urethane (1.5 g/kg) and put on its stomach on the heating board (37°C). The skin of the back was turned inside out. The cutaneous nerve branches innervating the rostral or caudal part of the back were exposed. The extracellular recording of their activities was simultaneously performed using two sets of bipolar electrodes of silver wire and AC bioelectric amplifiers (AB651; Nihon Kohden, Tokyo) with a band-pass filter of 1 kHz. Action potentials were counted for 60 min, using a data analyzing system with software to analyze the spike-height histogram (PowerLab/8s; AD Instruments Pty, Castle Hill, Australia). The opioid antagonist naltrexone (Sigma, St. Louis, MO, USA) was dissolved in physiological saline and injected subcutaneously. From 15 min after naltrexone injection, the behaviors and nerve activities were recorded for 60 min.

SPF mice rarely scratched the rostral back by their hind-paws, whereas CNV mice frequently scratched there; the average number of scratch bouts was 19 times greater in CNV mice than in SPF mice (Fig. 1A). Typical examples of the activity of cutaneous nerve innervating the rostral back are shown on Fig. 1B. The activity was as low as 0.1 Hz in SPF mice and markedly increased in CNV mice; the average frequency of the nerve of CNV mice was 8.6 times higher than that of SPF mice (Fig. 1C). To clarify the relation between the itch-associated behavior and the cutaneous nerve activity in NC mice, the data of each mouse in Figs. 1A and 1C were plotted in Fig. 1D. There was significant correlation between scratching and nerve firing (correlation coefficient $r = 0.964$, $P < 0.001$, Pearson product moment coefficient).

With regard to the caudal back, CNV, but not SPF, mice frequently bit there; the average number of bite bouts was 3.4 times greater in CNV mice than in SPF mice (Fig. 2A). Typical examples of the activity of cutaneous nerve innervating the caudal back are shown on Fig. 2B. The activity was as low as 0.1 Hz in SPF mice and markedly increased in CNV mice; the average frequency of the nerve of CNV mice was 5.4 times greater in CNV mice than in SPF mice (Fig. 2C). There was significant correlation between biting and nerve firing (correlation coefficient $r = 0.527$, $P < 0.05$, Pearson product moment coefficient).

Fig. 1. Spontaneous itch-associated scratching and the activity of cutaneous nerve innervating the rostral back of NC mice. Ten NC mice were kept under a specific pathogen-free (SPF) environment throughout the experiment (designated as SPF mice, open columns and symbols). Another ten mice were transferred to a conventional (CNV) environment at 4 weeks of age and kept together with the rodent mite for twelve days (designated as CNV mice, closed columns and symbols); nerve activity data from one CNV mouse was discarded because of experimental failure. A: Scratching of the rostral back. B: Typical data of spontaneous firing of nerve innervating the rostral back. C: Activity of nerve innervating the rostral back. D: Correlation between rostral-back scratching and activity of nerve innervating the rostral back. Scratching bouts were counted for 1 h. A,C: Values are means $\pm$ S.E.M. *$P < 0.05$ (Student’s t-test). D: Correlation coefficient $r = 0.964$, $P < 0.001$ (Pearson product moment coefficient).

Fig. 2. Spontaneous itch-associated biting and the activity of cutaneous nerve innervating the caudal back of NC mice. Spontaneous biting and nerve activity were observed in the same SPF and CNV NC mice as shown in Fig. 1. A: Biting of the caudal back. B: Typical data of spontaneous firing of nerve innervating the caudal back. C: Activity of nerve innervating the caudal back. D: Correlation between caudal-back biting and activity of nerve innervating the caudal back. Biting bouts were counted for 1 h. A and C: Values are means $\pm$ S.E.M. *$P < 0.05$ (Student’s t-test). D: Correlation coefficient $r = 0.527$, $P < 0.05$ (Pearson product moment coefficient).
In this series of experiments, the effects of naltrexone on
the itch-associated behaviors and cutaneous nerve activities
were examined in CNV mice. A subcutaneous injection of
naltrexone at a dose of 1 mg/kg produced 53% inhibition of
spontaneous scratching and 59% inhibition of spontaneous
biting (Fig. 3: A and B). On the other hand, the enhanced
activities of nerves innervating either the rostral or caudal
back in CNV mice were not affected by naltrexone at a dose
of 1 mg/kg (Fig. 3: C and D).

The marked increase of rostral-back scratching in CNV
mice was consistent in the previous observation (5, 6). The
strong correlation between the frequencies of spontaneous
scratching and nerve firing suggests that increase in the fir-
ing of the cutaneous nerve at least partly reflects an increase
in itch signals from the periphery, the skin of rostral back.
Similarly, significant correlation between the frequency of
biting and spontaneous nerve firing suggests that biting is at
least partly due to an increase in itch signals from the skin
of the caudal back. In contrast to the rostral part of the
body, there were no apparent cutaneous lesions in the
caudal part of the body (5). However, the marked increase
of the spontaneous firing of nerve innervating the caudal
skin manifests the presence of abnormal changes in dermal
conditions.

The frequency of spontaneous firing in SPF mice was
similar between cutaneous nerves innervating rostral and
caudal parts of the back, whereas in CNV mice, the activity
of nerve innervating the rostral back was apparently higher
than that of nerve innervating the caudal back. Apparent
dermatitis is localized in the rostral part of the body (5),
and the cutaneous barrier is disrupted in the rostral back,
but not in the caudal back (Y. Kuraishi et al., unpublished
observation). Such differences may be partly responsible
for the increase of spontaneous activity of nerve innervat-
ing the rostral back. In this context, the manipulation of
continuous disruption of cutaneous barrier increases sponta-
neous scratching (8). Changes in general skin conditions,
for example, due to allergy, may increase the nerve activity
and the disruption of cutaneous barrier may further aug-
ment the activity.

The principal result of this study is that naltrexone inhib-
ited itch-associated responses (scratching and biting) but
not the activities of cutaneous nerve. The results suggest
that the site of suppressive action of naltrexone on the itch-
associated behaviors is central but not peripheral. The idea
is supported by the result that rostral scratching and caudal
biting were inhibited by naltrexone to a similar extent. It is
also suggested that the opioid peptide in the central nervous
system, but not in the periphery, is involved in the sponta-
neous scratching and biting of CNV mice. The idea is
supported by findings that mice show scratching after an
intracisternal, but not intradermal, injection of opioids (11,
12). Pruritus of patients with cholestasis responds to the
opioid antagonist (3). Preproenkephalin mRNA is induced
in livers of rats with cholestasis (13) and opioid receptor-
mediated scratching is induced by an injection of plasma
from patients with pruritic cholestasis into the medullary
dorsal horn in monkey (14). Thus, even if the opioid
peptide is increased in the periphery, it is possible that it
acts on the central nervous system to elicit itching.

At present, endogenous mediators involved in these
phenomena of CNV mice remain unknown. Intradermal
injections of substance P and histamine, which elicit itch in
human subjects (15), do not elicit scratching in SPF mice
(5, 6). Serotonin produces scratching in SPF mice, but
spontaneous scratching of CNV mice is not significantly
inhibited by serotonin antagonists at the dose that inhibits
serotonin-induced scratching (5). Therefore, these endo-
genous substances may not play important roles in the
spontaneous scratching of CNV mice. Further study is
needed to elucidate the endogenous pruritogenic substances
involved in the chronic itching of NC mice.

In summary, it is suggested that NC mice have chronic
itching in the caudal part as well as rostral part of the body.
The cause of itch may be primarily peripheral, but the
mechanisms of itch in the caudal part of the body may not
be the same as those for the rostral itch. The site of anti-
pruritic action of the opioid antagonist naltrexone may not
be peripheral.
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