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Scratching and Wriggling Behaviors Induced by Compound 48/80 in Neonatal Rats

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Abstract: There is little information about itch-associated responses in neonatal rodents. We performed behavioral tests to elucidate itch-associated scratching and wriggling behaviors induced by compound 48/80 (C48/80) in 7- and 14-day-old rats. C48/80 (2.5 μg g^{-1} body weight per 5 μL) was s.c injected into the rostral part of the back of neonatal rats. Immediately after injection, their behavior was recorded for 30 min using a digital video camera under unmanned conditions. A series of scratching behaviors were counted as one bout of scratching. Total wriggling time was also monitored. Scratching behavior was observed in both ages, whereas wriggling behavior was seen only in 7-day-old rats. These results suggest that wriggling behavior is limited early in life and neonatal rats are available to reveal mechanisms of itch sensation and scratching behavior provoked by diseases specific to neonates or infants.

Key words: Itch, neonatal, scratching behavior, wriggling behavior

INTRODUCTION

Itching is an unpleasant cutaneous sensation that provokes the desire to scratch (Rothman, 1941). Itch sensation is transmitted from the peripheral pruriceptors to the Central Nervous System (CNS) via dorsal root ganglia and the spinal cord. This neural pathway exists in the spinothalamic tract and requires the activity of primary afferent neurons (Paus *et al*., 2006). Animals, including humans, can attenuate the itch sensation by scratching. However, over-scratching has negative influences on the skin. For example, skin barriers are damaged by scratching, allowing for bacteria invasion. When inflammatory factors are released, itch sensation is re-induced (Paus *et al*., 2006). This 'itch-scratch cycle' worsens serious dermatitis. Thus, inhibition of scratching behavior is considered beneficial for improving dermatitis and is a very important clinical treatment. There is no ultimate cure for itch since little is known about the mechanisms for feeling either itch sensation or scratching behavior.

Many patients suffer from diverse afflictions with itch sensation. For example, atopic dermatitis is a chronic inflammatory skin disease, usually affecting infants and children (Leung and Bieber, 2003) with itch being one of the most troublesome symptoms (Koblenzer, 1999). Atopic dermatitis also has a marked impact on quality of life. Patients with atopic dermatitis suffer from itch, sleep disturbance and the social stigma of a visible skin condition (Buys, 2007).

Scratching behavior is a response specific for pruritogen, but not algogen in mice (Kuraishi *et al*., 1995). Therefore, most researchers observe scratching behavior as a means to assess treatment effects. Compound 48/80 (C48/80), a condensation product of *N*-methyl-p-methoxy-phenethlamine with formaldehyde, also induces itch sensation since it works to degranulate mast cells, which contain histamine and several pruritogenic agents such as leukotriene B_4 (Andoh and Kuraishi, 1998). Controlling the scratching behavior is more difficult in neonates and infants, compared to adults. Furthermore, good models for scratching behavior in neonatal animals have been limited.

We therefore, investigated the behavioral changes after C 48/80 in neonatal rats at 2 ages.

MATERIALS AND METHODS

Male and female adult rats (Wistar strain purchased from Kyudo Co., Ltd., Saga, Japan) were kept at 23±1°C on a 12-h light/dark cycle (7:00 light on), having free access to a commercial diet (MF; Oriental Yeast, Tokyo, Japan) and water. The pups from these rats were used at age 7 and 14 days.

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Experimental procedures followed the guidelines for animal experiments in the Faculty of Agriculture and the Graduate Course of Kyushu University, as well as the Law No. 105) and Notification (No. 6) of the Government.

C48/80 (2.5 μg g^{-1} body weight per 5 μL, Sigma Chemical Co., St. Louis, MO, USA) was dissolved in saline as a pruritogenic agent and saline was used as a control. These agents were s.c injected into the rostral part of the back of neonatal rats. Immediately after injection each rat was put into an acrylic box composed of 4 cells. Their behavior was recorded for 30 min using a digital video camera under unmanned conditions. According to a previous study (Kuraishi *et al.*, 1995), mice scratched at pruritogenic agent injected sites with the hind paws when they probably experienced itch sensations. A series of scratching behaviors were counted as one bout of scratching. Total wriggling time was also monitored. A continuous scratching behavior was counted as one bout of scratching at 5-min intervals. Wriggling time was counted at 5 min intervals.

Data for behavioral tests were analyzed by repeated measure ANOVA. Differences were considered significant at $p<0.05$. Results are shown as means±S.E.M.

RESULTS

Figure 1 shows scratching behavior after s.c. C48/80 injection in neonatal rats. C 48/80 elicited itch-associated responses in neonatal rats ($F(1, 20) = 6.416$, $p<0.05$). Rats scratched the C 48/80 injected site with the hind paws at both ages. No other main effects and interactions were deemed significant.

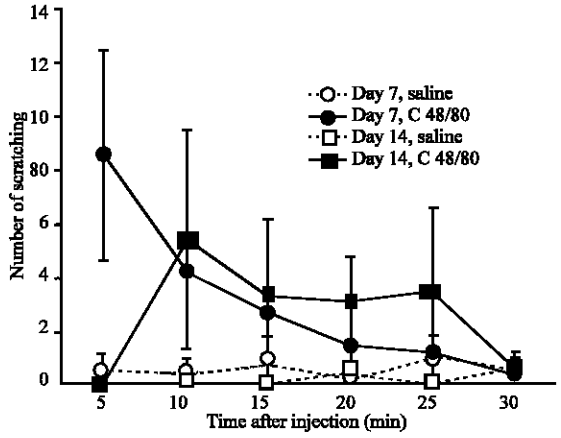

Fig. 1: Time course of scratching behavior after an s.c. injection of saline (n = 6 and 5) and C48/80 (n = 6 and 7) in 7- and 14-day-old rats, respectively. Values are expressed as mean±S.E.M.

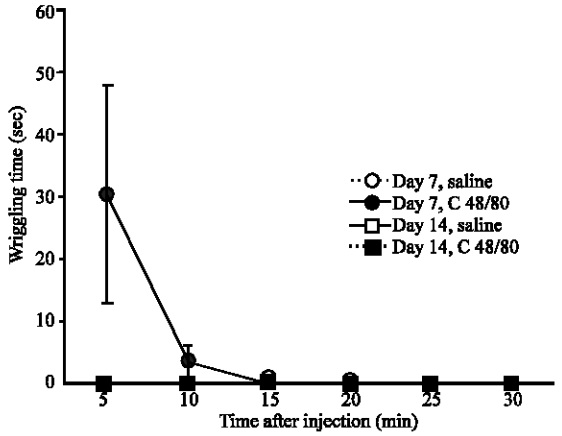

Fig. 2: Time course of wriggling behavior after an s.c. injection of saline (n = 6 and 5) and C 48/80 (n = 6 and 7) in 7- and 14-day-old rats, respectively. Values are expressed as mean±S.E.M.

Figure 2 gives wriggling behavior after s.c. C 48/80 injection in neonatal rats. A significant effect of time ($F(5, 100) = 2.939$, $p<0.05$) and significant interactions between time and day ($F(5, 100) = 2.939$, $p<0.05$), time and treatment ($F(5, 100) = 3.000$, $p<0.05$), or among time, day and treatment ($F(5, 100) = 3.000$, $p<0.05$) were detected. These results imply that wriggling behavior occurred with C48/80 injection in 7-day-old rats only.

DISCUSSION

The injection of C 48/80 clearly induced itch-associated responses, i.e., scratching and wriggling behaviors in neonatal rats, even at 7 days of age. Wriggling behaviors may be induced by pruritogens and limited to early life, since younger neonates are predicted to have undeveloped behavioral systems. It was reported that the function of hindlimbs was not sufficiently mature until the latter half of the second week (Altman and Sudarshan, 1975) and 20 day old rats had adult-like locomotor activities compared to 1-10 day old rats (Piggins and Merali, 1992b). These facts support the present observations, where wriggling may be an alternative response to scratching.

Most researchers used adult rats or mice for studies of itch. Accordingly, little information is available for itch-associated response in neonatal rodents. Scratching or grooming behavior was observed at 1-5 and 10 day old rats by s.c. administration of bombesin (Piggins and Merali, 1992a) and at 5-10 and 20 day old rats by intracerebroventricular injection of bombesin (Jackson and Kitchen, 1989). In the present study, it was clear that existing itch-associated response such as

scratching behavior (Kuraishi *et al*., 1995) was also caused by C 48/80 even in neonatal rats. Further, it was found wriggling behavior, a novel response to pruritogen, was limited early on in the neonatal stage and could be used as an indicator of increasing itch sensation. These results made it possible to use a neonatal rat model for itch-associated behavioral tests and neonatal rats may be utilized to reveal mechanisms of itch sensation and scratching behavior provoked by diseases specific to neonates or infants.

CONCLUSION

Itch-associated responses were caused by administration of C 48/80 in neonatal rats and wriggling behavior was observed in 7 day old rats only. Wriggling behavior may be an alternative response to scratching behavior, since younger neonates are predicted to have undeveloped behavioral systems.

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