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Itch-Specific C-fibers were not Destroyed by Neonatal Capsaicin Treatment in Rats

Y. Akimoto, D. Oikawa, S. Tsuyama and M. Furuse
Advanced Animal and Marine Bioresources,
Graduate School of Bioresource and Bioenvironmental Sciences,
Kyushu University, Fukuoka 812-8581, Japan

Abstract: Deafferentation can be achieved by neonatal capsaicin treatment in rats. We estimated that itchy sensation would be attenuated by neonatal capsaicin treatment, because itchy sensation is transmitted to the central nervous system via primary afferent C-fibers in the spinothalamic tract. To reveal neonatal deafferentation modify the scratching behavior induced by pruritogenic agents in rats, we treated neonatal rats with capsaicin to deafferent C-fibers and performed behavioral tests using pruritogenic agents. Deafferentation by capsaicin was successful, as evidenced by the decreased number of eye wipings induced by NaOH compared with the control rats. Histamine or compound 48/80, mast cell degranulationg agent, was injected subcutaneously as a pruritogenic agents and then scratching behavior was monitored over 30 min. No significant differences were detected between capsaicin-treated and control groups. These results suggest that neonatal deafferentation does not modify scratching behavior induced by pruritogenic agents in rats and itch-specific C-fibers are not destroyed by neonatal capsaicin treatment or deafferentation activates other itchy pathway.

Key words: Capsaicin treatment, deafferentation, itch, pruritogenic agent, scratching behavior

INTRODUCTION

Itch is defined as the unpleasant sensation attendant on the scratching drive. Itch sensation is transmitted from the peripheral pruriceptors to the Central Nervous System (CNS) via Dorsal Root Ganglia (DRG) and the dorsal horn of the spinal cord. This neural pathway exists in the Spinothalamic Tract (STT) and requires the activity of primary afferent C-fibers (Twicross *et al*., 2003; Ikoma *et al*., 2006; Paus *et al*., 2006). Animals, including humans, can attenuate the itchy sensation by scratching. However, over-scratching badly influences the skin condition. For example, skin barriers are damaged by scratching thus allowing bacteria to invade through the skin. When inflammatory factors are released, itchy sensation is re-induced (Paus *et al*., 2006). This "itch-scratching cycle" worsens atopic dermatitis or other serious dermatitis. Thus, inhibition of scratching behavior is considered beneficial for improving dermatitis and is thus very important for clinical treatment. However, there is no ultimate cure for itch since little is known about the mechanism for scratching behavior or feeling itchy sensation.

Histamine causes itchy sensation mainly in the periphery. Since, histamine-sensitive neurons were found, some researchers suggested the existence of itch selective primary afferent C-fibers (Schmelz *et al*., 1997; Andrew and Craig, 2001). However, itch specific C-fibers have not yet been found and the neural basis of the itchy sensation remain unclear (Schmelz, 2001).

Capsaicin is the pungent pain producing substance of chili pepper. Janscó *et al*. (1977) reported that the function of the chemosensitive primary sensory neurons was impaired irreversibly in rats pretreated with capsaicin at the neonatal stage and some of the small types of sensory ganglion cells were damaged. Later, it was reported that neonatal capsaicin pretreatment resulted in disruption of small DRG neurons and unmyelinated C-fibers and these influences of neonatal capsaicin treatment were permanent and showed no signs of unmyelinated fibers regeneration (Scadding, 1980; Fitzgerald, 1983; Hiura and Sakamoto, 1987; Hiura *et al*., 1990). Therefore, we estimated that itchy sensation would be attenuated by capsaicin-induced deafferentation, because itchy sensation is transmitted via unmyelinated C-fibers in the STT. To reveal neonatal deafferentation modify scratching behavior induced by pruritogenic agents in rats, we treated neonatal rats with capsaicin and performed behavioral tests using pruritogenic agents.

Corresponding Author: M. Furuse, Advanced Animal and Marine Bioresources,
Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan

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) and had free access to a commercial diet (MF; Oriental Yeast, Tokyo, Japan) and water. The pups from these rats were reared in our laboratory and pretreated with capsaicin or vehicle in the first 48 h of life. Experimental procedures followed the guides 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.

According to previously studies (Janscó *et al*., 1977; Hill *et al*., 1991; Van de Wall *et al*., 2006), animals were treated with capsaicin (50 mg kg^{-1}) to blockade C-fibers or with the vehicle as a control. Capsaicin (8-methyl-N-vallinyl 6 nonenamide, Sigma Chemical Co., St. Louis, MO, USA) was dissolved in vehicle consisting ethanol, Tween 80 (Sigma Chemical Co., St. Louis, MO, USA) and physiological saline (1:1:8 (v/v/v)). Drugs were given to neonatal rats anesthetized with diethyl ether (Wako Pure Chemical Industries, Ltd., Osaka, Japan) within the first 48 h after birth by subcutaneous (s.c.) injection. After treatment, all pups were returned to their dam's care until weaning age (21-23 days old).

The eye-wiping test was used for investigating the reduction of corneal chemosensitivity and assessing the effect of capsaicin treatment. Compared with control rats, capsaicin treated rats are usually insensitive to noxious stimuli. Farazifard *et al.* (2005) described that the reduction of chemosensitivity confirms the effective depletion of C-fiber afferents, because the information of chemosensitivity is mainly transmitted through C-fibers. This test was done using 1% NaOH as a test solution in both capsaicin-treated rats and vehicle-treated rats at the age of 5 weeks. According to the method of Lutz *et al.* (1998), a few drops of the test solution were applied and the number of eye-wiping for 10 sec counted after the application. Normally control rats aggressively wipe their eyes to remove stimulus. Capsaicin treatment was considered to be successful when capsaicin-treated rats scarcely wiped, since capsaicin treated rats probably were insensitive to noxious stimuli by blocking C-fibers.

Histamine (3 mg kg^{-1}, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and compound 48/80 (C48/80), a condensation product of N-methyl-p-methoxy-phenethlamine with formaldehyde, (2.5 mg kg^{-1}, Sigma Chemical Co., St. Louis, MO, USA) were dissolved in saline as pruritogenic agents used in Experiment 1 and Experiment 2, respectively.

Pruritogenic agents were s.c. injected into the rostral part of the back of capsaicin-treated rats and vehicle-treated rats. Immediately after injection, each rat was put into an acrylic box composed of four cells. Their scratching behavior was recorded for 30 min using a digital video camera under unmanned conditions. According to the previous study (Kuraishi *et al*., 1995), mice scratched pruritogenic agent injected sites with the hind paws when they probably feel itchy sensations. A series of scratching behaviors were counted as one bout of scratching.

We used the same rats in Experiments 1 and 2. To avoid the effect of Experiment 1, Experiment 2 was carried out 10 days after Experiment 1. No clear problem with these experiments was observed in Experiment 2, since histamine used in Experiment 1 caused acute itchy sensation. All conditions for behavioral test in Experiment 2 were same as Experiment 1.

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

RESULTS

Neonatal capsaicin treated rats showed significantly less eye wiping compared with control rats in both eyes. Left eye: capsaicin treated rats wiped 3.9±0.9 while control rats wiped 10.1±1.1 per 10 seconds ($F(1,13) = 0.701$, $p<0.001$). Right eye: capsaicin treated and control rats wiped 1.9±0.52 and 11.7±0.87 ($F(1,13) = 0.406$, $p<0.0001$), respectively.

Experiment 1: As shown in Fig. 1, all rats scratched the histamine injected site with the hind paws with or without capsaicin treatment ($F(1,13) = 0.831$, $p>0.05$). A continuous scratching behavior was counted as one bout of scratching at 5 min intervals. The number of scratching episodes among each interval was significantly ($F(5,65) = 3.241$, $p<0.05$) different. No significant ($F(5,65) = 0.769$, $p>0.05$) interaction was detected between capsaicin treatment and time after injection. The number of scratching peaked around 10-15 min in control rats. However, we could see that capsaicin-treated rats presented stronger and longer response for about 15 min as compared with control rats.

Experiment 2: Figure 2 shows the effects of C48/80 on the number of scratching episodes in rats with or without capsaicin treatment. As observed in Experiment 1, no significant ($F(1,13) = 0.121$, $p>0.05$) effect was detected

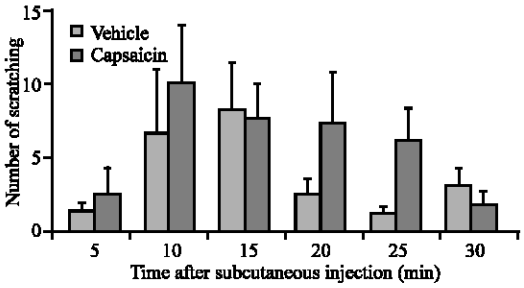

Fig. 1: Effect of neonatal capsaicin treatment on after an s.c. injection of histamine (3 mg kg^{-1}) induced scratching behavior. Rats treated with capsaicin (n = 8) and vehicle (n = 7). Results are expressed as means±S.E.M

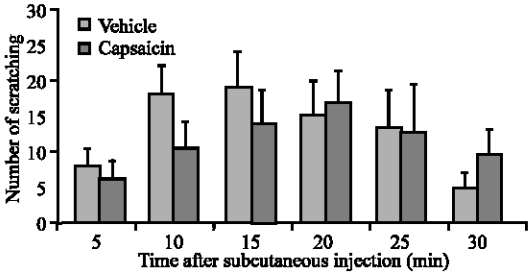

Fig. 2: Effect of neonatal capsaicin treatment on after an s.c. injection of C 48/80 (2.5 mg kg^{-1}) induced scratching behavior. Rats treated with capsaicin (n = 8) and vehicle (n = 7). Results are expressed as means±S.E.M

between control and capsaicin treated rats. Vigorous scratching behavior was observed over time ($F(5,65) = 2.724$, $p<0.05$), but no significant ($F(5,65) = 0.760$, $p>0.05$) interaction was detected. It seemed that the tendency in response of capsaicin-treated rats was delayed to C48/80 treated rats.

DISCUSSION

From the results of the eye-wiping test, it appeared that rats treated with capsaicin suffered from the reduction of chemosensitivity and it was confirmed the effective depletion of C-fiber afferents (Fitzgerald, 1983). Since, itch sensation is transmitted through C-fibers (Schmelz *et al*., 1997; Andrew and Craig, 2001; Twicross *et al*., 2003; Ikoma *et al*., 2006; Paus *et al*., 2006), we presumed that the scratching behavior induced by histamine would be attenuated by capsaicin treatment. However, vigorous scratching behavior was observed in both groups and no significant differences were detected in the number of scratches. We further confirmed the effect of mast cell degranulation by C48/80, since, mast cells contain several pruritogenic agents such as histamine and leukotriene B_4 (Andoh and Kuraishi, 1998). However, the effect of C48/80 on scratching behavior was similar between control and capsaicin treated rats as observed with histamine. These results suggest that neonatal deafferentation does not modify scratching behavior induced by pruritogenic agents in rats.

Nevertheless, it was reported that neonatal capsaicin treatment caused 85-95% loss of C-fibers in lumbar dorsal roots (Nagy *et al*., 1981), itch-associated scratching behavior was observed in the present study. Furthermore, it was stated that a resistant 5% of C-fibers always remains in the dorsal root (Fitzgerald, 1983). Therefore, it was predicted that itch-specific C-fibers were not completely destroyed by neonatal capsaicin treatment.

Conversely, it could speculate that the sensitivity of itchy sensation was potentially enhanced by blocking the primary afferent C-fibers. Opioid peptides such as morphine are related to itchy sensation (Ballantyne *et al*., 1988; Toida *et al*., 1997; Kuraishi *et al*., 2000). In neonatal rats treated with capsaicin, morphine induced extreme scratching behavior and this response was inhibited by naloxone, a morphine antagonist. However, morphine did not have a significant effect in vehicle treated rats (Thomas *et al*., 1994). This was suggested the possibility that deafferentation caused by neonatal capsaicin treatment activates the opioidergic pathway. However, there are no clear evidence for capsaicin treated rats and itchy sensation.

Therefore, further researches are required to elucidate the relationship between neonatal capsaicin treatment and itch-associated scratching behavior elicited by pruritogenic agents.

CONCLUSION

Neonatal capsaicin treatment does not modify scratching behavior elicited by peripherally administered pruritogenic agents. There are possibilities that itch-specific C-fibers are not destroyed by neonatal capsaicin treatment or deafferentation activates other itchy pathway.

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