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Sex Differences in Scratching Behaviors Induced by Intradermal Injections of Pruritogenic Chemicals in C57BL/6 Mice

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ABSTRACT

Pruritus is an individual unpleasant sensation of human sensory nervous system. In the physiological condition it excerts a self-protective mechanism to protect the skin against external harmful agents. Pruritoceptive itch is also a major symptom of skin diseases and a common reason for consulting a dermatologist in clinic. It has been well known that both histamine-dependent and histamine-independent pathways mediate acute and chronic itch sensations. Previous studies have showed common neural pathways partially shared by itch and pain sensations, and significant sex differences in pain sensation. However, sex difference in itch sensation has not been given too much attention as the majority of itch studies were done in male mice or rats till now. In the present study, we compared the scratching behaviors induced by pruritogenic agents in male and female C57BL/6 mice. The results showed that both male and female exhibited scratching behaviors in response to the intradermal injection of histamine-dependent and histamine-independent pruritogenic chemicals. Moreover, the number of scratching behaviors in response to compound 48/80 and chloroquine were significantly higher in female. These results suggested that sex differences occurred in histamine-dependent compound 48/80-induced and histamine-independent chloroquine-induced itch sensations, but not in histamine-independent SLIGRL-NH_2-induced itch sensation.

Keywords: Scratching; Pruritus; Sex Difference; Compound 48/80; Chloroquine; SLIGRL-NH_2

1. Introduction

Pruritus, or itch, is defined as an unpleasant sensation that elicits the desire or reflex to scratch [1]. Pruritoceptive itch, or acute itch, serves as a physiological self-protection as other cutaneous sensations such as pain, touch and etc., to prevent the skin from harmful external enviroment. Pruritoceptive itch originates peripheral skin and is transmitted by slow-conducting subsets of unmyelinated C nerve fibres [2]. Chronic itch is also a bothersome symptom in some skin and systemic diseases, for example, atopic dermatitis, cholestasis and etc. [2-4]. In addition, acute or chronic itch is also one adverse side-effect of many drugs including antimalarial drug chloroquine, opioid and so on [5]. It has been identified that both histamine-dependent and histamine-independent pathways mediate acute and chronic itch sensations [6]. Compound 48/80 which is known to elicit itch by degranuating mast cells to release histamine has been proved to be a more reliable pruritogen in the study of itch [7]. The protease activated receptor 2 (PAR2) agonist SLIGRL-NH_2 has been recently proved to be a histamine-independent pruritogen [8,9]. For a long time, it is considered that itching and pain have neural pathways partially [6]. Low intensity stimulation of unmyelinated polymodal C fiber results in sensation of pruritus whereas high intensity stimulation causes pain [1]. Itch can be suppressed by pain-evoking mechanical or thermal stimuli. Patients with spinally administered μ-opioid agonists frequently experience itch [10]. Recently, gastrin-releasing peptide receptor (GRPR) and MrgprA3, or one member of a family of G protein-coupled receptors, are identified to mediate itch sensation along itch signal-

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ing pathways [11,12]. Thus, pruritus is not merely a submodality of pain, but also an individual sensation of our sensory nerve system.

Sex-associated differences in the pain perception and its modulation have been widely confirmed in humans and animals as well. Female tend to exhibit more pain sensitivity, lower pain threshold and tolerance than male [13-16]. However, sex-associated differences in itch sensation and its modulation remain unclear. Despite it was reported that female displayed a higher chronic scratching behavior than male in a model of autoimmune disease of MRL/lpr mice [17], and it was also observed that female were more severely affected by pruritus among chronic urticaria patients [10], the occurrence of pruritus was not correlated with sex of the patients in the clinical research of patients undergoing hemodialysis [11]. Therefore, sex-associated difference in itch sensation is still unclear. In addition, the majority of itch studies were done in male mice or rats nowadays. Thus, our study was designed to ascertain the sex-associated difference in itch sensation induced by intradermal injections of pruritogenic chemicals compound 48/80, SLIGRL-NH_2 and chloroquine as well in C57BL/6 mice of both sexes.

2. Materials and Methods

2.1. Animals

Both male and female C57BL/6 mice (6 - 8-week-old) were purchased from the Fourth Military Medical University. All experiments were carried out according to the guidelines of the Institutional Animal Care and Use Committee of the Fourth Military Medical University.

2.2. Itch Behaviors

The mice were allowed to acclimate to the observation room for one week. Three days before experiments, a 1.5 cm diameter circular area was shaved at the back of the neck where intradermal injections were given. Each mouse was habituated to Plexiglas observation cylinders (9 × 9 × 13 cm) at least 30 minutes per day for three days prior to experiments. Pruritogenic compounds (*i.e.*, compound 48/80, chloroquine and SLIGR L-NH2 as well) were subcutaneously injected into the nape of the neck at a volume of 50 μl after acclimatization. Scratch- ing behavior was observed for 30 minutes at 5-minute intervals. Video cameras located above the glass floor and recorded activity for the next 30 minutes. Digital video files were later scored for the total number of scratching strokes to the injected area during the 30 minutes recorded. A bout of scratching was defined as continuous scratch movements with hind paws directed at the area around the injection site. Scratching behavior was quantified by recording the number of scratching bouts at 5-minute intervals over the 30-minute observation period. One scratch was considered to be a lifting of the hind limb towards the shaved area and then a replacing of the limb back to the floor or licing the hind limb, regardless of how many scratching strokes took place between those two movements [9,12,18]. As to intradermal injections, the following drugs were dissolved in sterile saline and administered at a volume of 50 μl: compound 48/80 (100 μg/50 μl), SLIGRL-NH_2 (100 μg/50 μl), chloroquine (200 μg/50 μl).

2.3. Data Analysis

Data were presented as means ± sem. Statistical analysis was performed with repeated measure analysis of variance or Student's unpaired t-test. $P < 0.05$ was considered statistically significant.

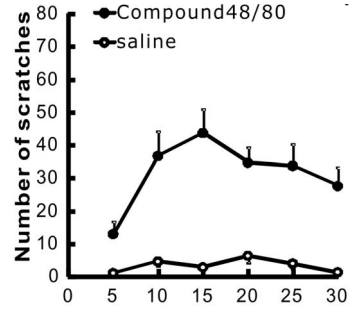
3. Results

3.1. Scratching Behaviors Induced by Intradermal Injections of Pruritogenic Chemicals in Male C57BL/6 Mice

Compared with the injection of saline (n = 13), compound 48/80 (100 μg/50 μl，n = 10) induced scratching behaviors in male C57BL/6 mice, which often began seconds after the intradermal injection and was sustained within 30 minutes (**Figures 1(a)** and **1(b)**). After compound 48/80 was injected, the scratching behavior was peaked at 15 minutes in male mice. The Analysis of total scratching numbers in the 30 minutes showed a signifycant scratching effect after the injection in male C57BL/6 mice (Student's unpaired t-test. $P < 0.01$, **Figure 1(b)**). Similarly, two histamine-independent pruritogenic agents, chloroquine（200 μg/50 μl, (**Figures 1(c)** and **1(d)**) and an agonist of the prote-ase-activated receptor 2 (PAR2), or SLIGRL-NH_2 (100 μg/50 μl, **Figures 1(e)** and **1(f)**), also induced signifycant scratching behaviors, which often began in several seconds after the intradermal injection and was sustained within 30 minutes. Analysis of total scratching numbers in the 30 minutes showed both chloroquine (n = 12) and SLIGRL-NH_2 (n = 12) induced significant itchy scratching behaviors after the injection (Student's unpaired t-test, $P < 0.01$).

3.2. Scratching Behaviors Induced by Intradermal Injections of Pruritogenic Chemicals in Female C57BL/6 Mice

Compared with the injections of saline (n = 12) in female C57BL/6 mice, compound 48/80 (n = 12) induced scratching behavior, which often began seconds after the intradermal injection and was sustained within 30 minutes (**Figures 2(a)** and **2(b)**). Analysis of total scratching numbers in the 30 minutes after the injection showed a



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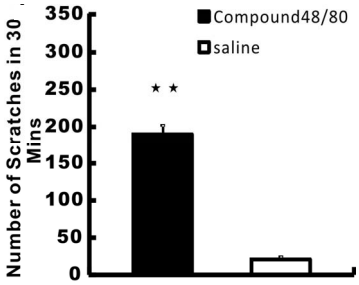

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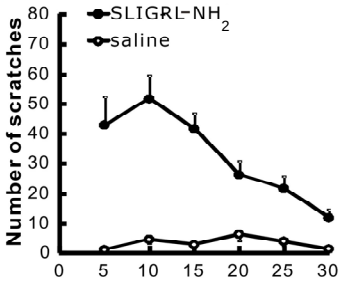

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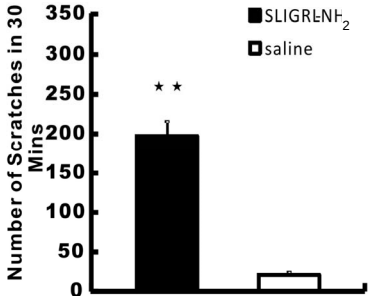

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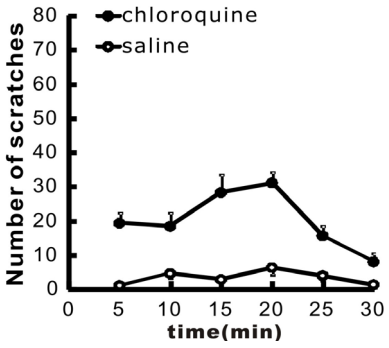

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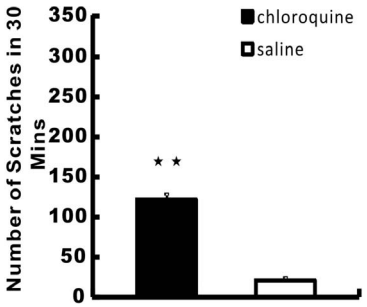

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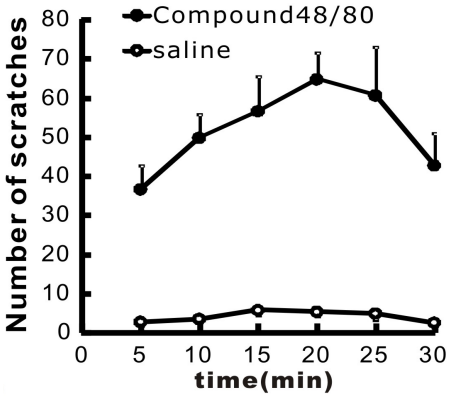
Figure 1. Scratching behaviors induced by the intradermal injection of pruritogenic compounds in male C57BL/6 mice. (a) The time course of every 5 minutes of histamine-dependent compound 48/80-evoked significant scratching behaveiors during 30 minutes in male mice (n = 10) (repeated measure analysis of variance, $P < 0.01$, saline group, n = 13); (b) The total number of scratches of histamine-dependent compound 48/80 during 30 minutes in male mice (n = 10) (Student's unpaired t-test, $P < 0.01$, saline group, n = 13); (c) The time course of every 5 minutes of histamine-independent chloroquine-evoked significant scratching behaviors during 30 minutes in male mice (n = 12) (repeated measure analysis of variance, $P < 0.01$, saline group, n = 13); (d) The total number of scratches of histamine-independent chloroquine during 30 minutes in male mice (n = 12) (Student's unpaired t-test, $P < 0.01$, saline group, n = 13); (e) The time course of every 5 minutes of histamine-independent SLIGRL-NH_2-evoked significant scratching behaviors during 30 minutes in male mice (n = 12) (repeated measure analysis of variance, $P < 0.01$, saline group, n = 13); (f) The total number of scratches of histamine-independent SLIGRL-NH_2 during 30 minutes in male mice (n = 12) (Student's unpaired t-test, $P < 0.01$, saline group, n = 13).

significant scratching effect in female C57BL/6 mice (**Figure 2(b)**). Similarly, two histamine-independent pruritogenic agents, chloroquine and an agonist of the protease-activated receptor 2 (PAR2) also induced signifycant scratching behaviors, which often began in several seconds after the intradermal injection and was sustained within 30 minutes. After compound 48/80 was injected, the scratching behavior of female mice was peaked at 20 minutes. Analysis of total scratching numbers in the 30 minutes showed chloroquine (n = 13, Student's unpaired t-test, $P < 0.05$, **Figures 2(c)** and **(d)**) and SLIGRL-NH_2 (n = 12, Student's unpaired t-test, $P < 0.01$, **Figures 2(e)** and **(f)**) induced significant scratching behaviors after the injection.

3.3. Significant Scratching Behaviors Induced by Intradermal Injections of Compound 48/80 and Chloroquine, but Not Sligrl-NH_2, in Female C57bl/6 Mice

After the injection of compound 48/80, the scratching behavior was peaked at 20 minutes in female mice. The scratching behavior induced by intradermal injection of compound 48/80 (100 mg/50 ml) was significantly



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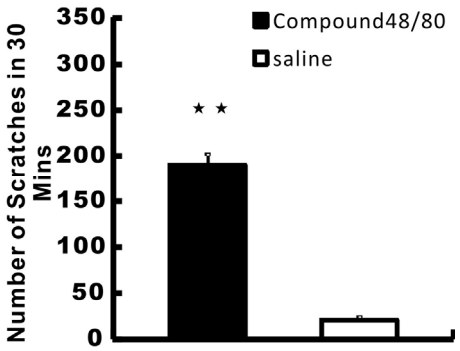

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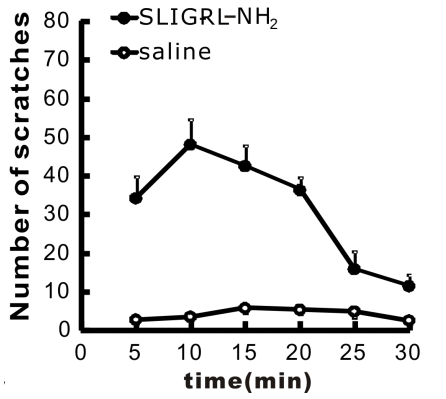

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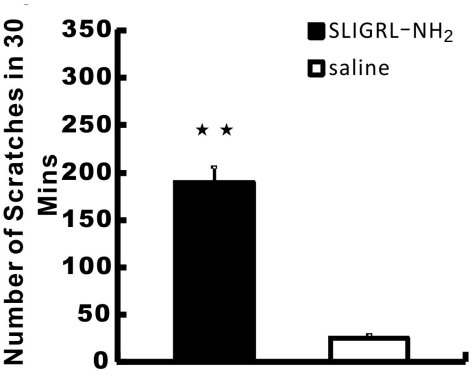

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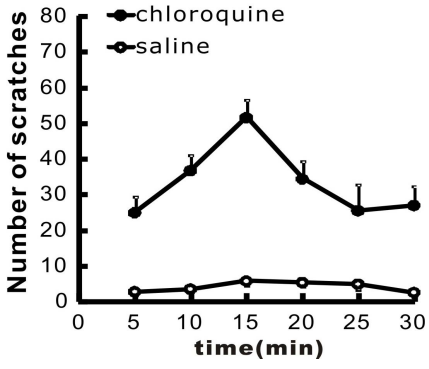

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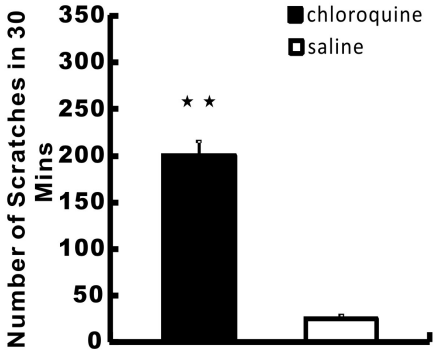

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Figure 2. Scratching behaviors induced by intradermal injection of pruritogenic chemicals in female C57BL/6 mice. (a) The time course of every 5 minutes of histamine-dependent compound 48/80-evoked significant scratching behaviors during 30 minutes in female mice (n = 12) (repeated measure analysis of variance, $P < 0.01$, saline group, n = 12); (b) The total number of scratches of histamine-dependent compound 48/80 during 30 minutes in female mice (n = 12). (Student's unpaired t-test, $P < 0.01$, saline group, n = 12); (c) The time course of every 5 minutes of histamine-independent SLIGRL-NH_2-evoked significant scratching behaviors during 30 minutes in female mice (n = 12) (repeated measure analysis of variance, $P < 0.01$, saline group, n = 12); (d) The total number of scratches in female mice after the injection of pruritogen or saline in a period of 30 min. Compound 48/80 and SLIGRL-NH_2 both induced significantly higher number of scratching behaviors than saline group (Student's unpaired t-test, $P < 0.01$); (e) The time course of every 5 minutes of histamine-independent chloroquine-evoked significant scratching behaviors during 30 minutes in female mice (n = 12) (repeated measure analysis of variance, $P < 0.01$, saline group, n = 12); (f) The total number of scratches of histamine-independent chloroquine during 30 minutes in female mice (n = 13) (Student's unpaired t-test, $P < 0.01$, saline group, n = 12).

higher in female mice (n = 12) compared with male mice (n = 10, repeated measure analysis of variance, $P < 0.01$, **Figures 3(a)** and **3(b)**). The scratching behaviors induced by intradermal injection of histamine-independent pruritogenic agent chloroquine was significantly higher in female mice (n = 12) compared with male mice (n = 10, repeated measure analysis of variance, $P < 0.01$, **Figures**

3(c) and **3(d)**). The scratching behavior induced by intradermal injection of PAR2 agonist SLIGRL-NH_2 in male mice (n = 12) showed no significant difference from female mice (n = 12, repeated measure analysis of variance, $P > 0.05$, **Figures 3(e)** and **3(f)**).

4. Discussion

The present study showed that the sex-associated difference in itch sensation induced by intradermal injections of pruritogenic chemicals compound 48/80 and chloroquine as well in C57BL/6 mice.

Tons of evidence demonstrate that sex-associated differences are universal in the pain perception and its modulation. Female exhibits not only more pain sensitivity, lower pain threshold but also tolerance in experimental model and clinical patients as well [14-16,19,20]. Moreover, electrophysiological and imaging studies revealed that different central neurophysiological activeties to noxious stimuli may contribute to sex-associated differences in the perception and modulation of pain. Here,

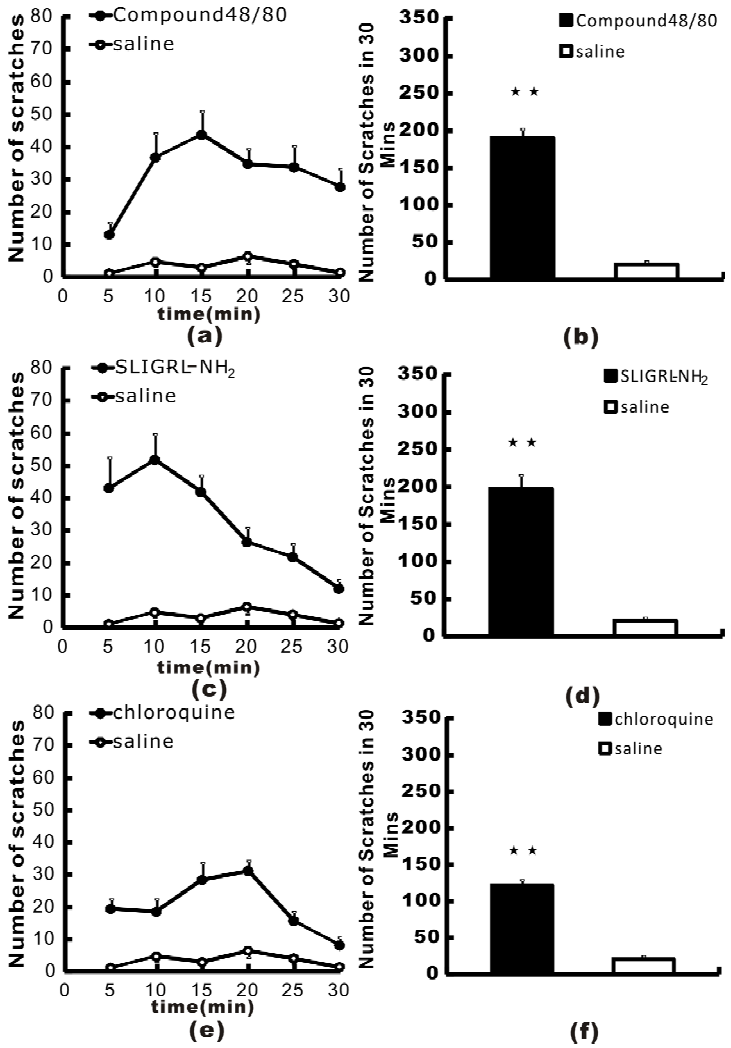

Figure 3. Significantly higher scratching behaviors induced by intradermal injection of compound 48/80 and chloroquine in female mice. (a) After the injection of compound 48/80, the scratching behavior was peaked at 20 minutes in female mice, whereas the peak was arised at 15 minutes after injection in male mice (repeated measure analysis of variance, $P < 0.01$); (b) The scratching behavior induced by intradermal injection of compound 48/80 (100 mg/50 ml) was significantly higher in female mice (n = 12) compared with male mice (n = 10, Student's unpaired t-test, $P < 0.01$); (c) The scratching behaviors induced by intradermal injection of chloroquine (200 mg/50 ml) in male and female mice (n = 12, repeated measure analysis of variance, $P < 0.01$); (d) The scratching behavior induced by intradermal injection of chloroquine was significantly higher in female mice (n = 12) compared with male mice (n = 10, Student's unpaired t-test, $P < 0.01$); (e) The scratching behavior induced by intradermal injection of PAR2 agonist SLIGRL-NH_2 in male mice (n = 12) showed no significant difference from female mice (n = 12, repeated measure analysis of variance, $P > 0.05$); (f) The total number of scratches was not significantly different after the injection of SLIGRL-NH_2 in a period of 30 minutes between female and male mice (Student's unpaired t-test, $P > 0.05$).

we presented evidence that both male and female exhibited scratching behaviors in response to both hista [21,22]. Compared to pain sensation, it is still poorly known about sex-associated differences in itch sensation mine-dependent and histamine-independent pruritogenic chemicals, and sex-associated differences in itch sensation induced by intradermal injections of histamine-dependent pruritogenic compound 48/80 and histamine-independent pruritogenic chloroquine. Histamine is one well characterized itch mediator till now. In clinic, female displayed larger wheal responses than male after administration of histamine by iontophoresis to human subjects [23]. It was reported that female had higher itching scores following burns, especially at 3 months postburn [24]. Female patients were also more severely affected by pruritus among the sufferer from chronic urticaria [10]. In experimental studies, there were a few studies showed that female had more significant scratching behaviors induced by the injection of chloroquine [25] and that female MRL/lpr mice displayed higher chronic scratching behaviors in a pruritic animal model [17]. All these results indicated that there may exist sex-associated differences in itch sensation. Our present study further provided the evidence that female mice exhibited higher scratching scores in response to intradermal injections of histamine-dependent pruritogenic compound 48/80 and histamine-independent pruritogenic chloroquine. Surprisingly, there was no sex-assocaited difference in itch sensation induced by the intradermal injection of histamine-independent pruritogenic PAR2 agonist. Our results indicate different mechanisms of chloroquine and PAR2 agonist might contribute to histamine-independent itch sensation. Previous study showed that histamine receptor H1 (H1R) densities of the central histaminergic neurons in female were higher than male [26]. It is likely that different distribution of histamine receptor H1 in the male and female may contribute to the sex-associated differences in itch senstion induced by histamine-dependent pruritogenic compound 48/80 either through central or peipheral pruritus pathways. Recently, it is reported that sex hormone may accelerate the decay of the histamine-induced peak calcium response in cultured human umbilical vein endothelial cells [27]. Sex hormones also modulated the intracellular Ca^{2+} response induced by histamine in cultured airway smooth muscle from female patients [28]. Thus, it is very likely that sex hormones contribute to the sex-associated differences induced by histamine dependent pruritogenic compound 48/80. But this is not able to explain the reason that the sex-associated differences induced by histamine-independent pruritogenic chloroquine.

Furthermore, our results showed that histamine-independent pruritogenic chloroquine induced more itch sensation in female mice. This is in line with that of Green's group [25]. It indicated that other endogenous and exogenous factors such as sex hormones, and genetic factors might be involved in sex related differences in itch sensations. The estrous cycle is a short-term, 4 - 5-day period, in mice according to vaginal cytology [29]. In clinic, estrogen sensitive women is easy to have estrogen dermatitis with pruritus [30]. It indicated that estrogen cycles might significantly affect pruritus behaviors. Thus, the precise analysis of influence of estrous cycle on scratching behaviors remained to be further investigated.

In conclusion, the present study demonstrates that the sex differences occurred in itch sensation induced by intradermal injections of histamine-dependent pruritogen compound 48/80 and histamine-independent prurito- gen chloroquine. Investigation into the impact of sex differences in itch sensation may enhance our understanding of the itch transmitting system.

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