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5-HT3 receptors antagonists reduce serotonin-induced scratching in mice

Running title: 5-HT3 receptors mediate serotonin-induced scratching

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ABSTRACT

Serotonin (5-hydroxytryptamine, 5-HT) acts as a pruritogen in humans and animals, but the mechanisms of action through that serotonin induces itch-response have not been extensively discovered. In our study, we attempted to investigate the role of 5-HT3 receptors in scratching behavior due to intradermal serotonin injection. Intradermal injection of serotonin (14.1–235 nmol/site) into the nape of the neck of mice was performed to elicit itch. Scratching behavior was evaluated by measuring the number of bouts during 60 minutes after injection. We evaluated the effect of intraperitoneal pretreatment with ondansetron and tropisetron (0.1, 0.3, and 1 mg/kg) on itch induced by serotonin. Also, intradermal ondansetron and tropisetron at doses 50, 100, and 200 nmol/site were concurrently administrated with serotonin. Serotonin produced a significant enhancement in scratching at dose 141 nmol/site. Concurrent administration of ondansetron (50, 100, and 200 nmol/site) and tropisetron (100 and 200 nmol/site) with serotonin reduced scratching activity compared to the animals that only received serotonin. Also, pretreatment with intraperitoneal ondansetron and tropisetron (0.3 and 1 mg/kg) 30 min before serotonin attenuated the itch response. We showed that the scratching induced by intradermal serotonin is mediated by 5-HT3 receptors subtype. It can be concluded that 5-HT3 may play a role in mediating serotonin-associated itch responses, and we introduce 5-HT3 receptors as possible targets for antipruritic agents.

Key words: Scratching; Serotonin (5-HT); 5-HT3 antagonists; Mice

ABBREVIATIONS

5-hydroxytryptamine (5-HT)

Intradermal (i.d.)

Intraperitoneal (i.p.)

One-way analysis of variances (ANOVA)

Protease activated receptor 2 (PAR2)

Standard error of mean (S.E.M.)

Subcutaneously (s.c.)

Transient receptor potential cation channel subfamily V member 1 (TRPV1)

INTRODUCTION

Itch (pruritus) is an unpleasant sensation that provokes the desire to scratch [1]. Pruritus may be seen during inflammation, cancer, metabolic diseases, infection, psychiatric disorders, drug use and stress [2]. Itch is the main symptom of not only cutaneous diseases, but also some systemic diseases like cholestasis and renal failure [3]. Also, it seriously affects quality of life of patients [4]. The precise mechanisms and mediators involved in most pruritic diseases are unclear at present. There are a variety of known itch-inducing mediators, including amines, neuropeptides, opioids, eicosanoids, etc [5]. Some mediators such as serotonin (5-hydroxytrypyamine, 5-HT) are said to be involved in originating the itch sensation [5]. Serotonin produces the itch sense when intradermally (i.d) or subcutaneously (s.c) applied to the human and rodent skin [6,7].

The serotonergic pathway may play a role in the perception of itch [1]. It has been proposed that some diseases including cholestasis, polycythemia vera, eczema, psoriasis and uremic disease induce itch through the serotonergic pathway [8-10]. However, the mechanisms through which intradermal serotonin induces pruritus have not been absolutely discovered. Serotonin acts on various receptor subtypes [11]. Past studies suggested that the pruritogenic effect of intradermal serotonin is related to its 5-HT2 receptor subtype in mice and rats [12]. On the other hand, 5-HT3 receptor antagonist administrations in patients reduced the itch due to cholestasis and uremic disease [13-15]. Therefore, it was concluded that ondansetron, a 5-HT3 antagonist, could elicit anti-pruritic effect against cholestasis, uremia, and opioid-induced itch [9,14,16]. Serotonin type 3 (5-HT3) receptor antagonists have been reported as an effective, safe, and well-tolerated therapeutic principle for some types of pruritus [17]; but it has not been cleared whether ondansetron and tropisetron which are high and potent antagonists of 5HT3 receptors [18] are capable of controlling the itch following intradermal serotonin injection. Advances in dermatology and neurology research have led to a credible realization of pruritus basis. Although antihistamines are standard treatments for itch, they are not beneficial enough in lessening pruritus in various conditions [5, 19]. Therefore, a better comprehension of itch is assumed to be an apparent importance from a therapeutic point of view. Thus, in this study we aimed to evaluate the serotonin-associated itch response in mice and the involvement of 5-HT3 receptors in development of this sense.

MATERIALS AND METHODS

Animals

Male NMRI mice (24–30 g, 6-10 w, Pasteur Institute) were used during our study. The animals were housed in a normal room temperature (25 ± 1°C) and a regular light/dark cycle. Food and water was available for animals ad libitum. All behavioral experiments were accomplished between 12:00 and 17:00 h. All groups consisted of 6-9 animals and each animal was used only once. Experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication #80-23) and institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS).

Reagents

Drugs used in our study were serotonin hydrochloride, ondansetron, and tropisetron (Sigma, St. Louis, USA). Serotonin was dissolved in physiological saline solution and was administered through intradermal injection. Ondansetron and tropisetron, 5-HT3 receptor antagonists [20], were also dissolved in saline and administered in a volume of 10 ml/kg for intraperitoneal (i.p) and 50µl/ site for i.d administration.

Administration procedures

Serotonin was injected intradermally into the rostral part of the back (inter scapular level). The syringes 24-25G insulin injection needles were used for the injections. Two days before the experiments, a 1.5 cm diameter circular district at the nape of the neck was shaved. Immediately after the injection, mice were placed in separate cages for video recording of scratching activity.

Ondansetron and tropisetron were administered i.p 30 minutes before serotonin injection, or i.d concurrently with serotonin.

Behavioral experiments

We measured the scratching behavior of animals as described in past reports. Briefly, the behaviors of mice were recorded for 60 minutes instantly after intradermal injection for observing the scratching behavior in absence of experimenter. Mice were held in private small Plexiglas chambers (10 ×10×10 cm) during the experiments. A vacuum line pulled ambient air through the chamber at a rate of 300 ml/min. Scratching was defined as motoric behavior when the hind paws moved up to the site of injection and touched the shaving area at least once. Only scratching at the site of injection was considered. A pause of more than 1 sec was defined as the end of a scratch series. Movements with the forelimbs were regarded as grooming and not scored as scratching activity.

Experiment design

After evaluating the effect of intradermal serotonin in scratch induction, we performed two experiments to examine the possible role of 5-HT3 receptor antagonists in induction of scratching via serotonin. In the first part, we examined the possibility of anti-pruritogenic properties of ondansetron and tropisetron by injecting these drugs at doses 50, 100, and 200 nmol/site intradermally to animals. In the second part, we evaluated the effect of treatment with intraperitoneal ondansetron and tropisetron (0.1, 0.3, and 1 mg/kg) on scratching behavior in mice. The antipruritogenic effect of these doses was examined in intact animals as well as 5-HT-injected mice. Saline (10 ml/kg and 50µl/ site) was injected into control groups to rule out the

effect of saline administration on animals' behaviors. These doses were adapted from our pilot studies and past reports (6).

Statistical analysis

Data are expressed as mean ± S.E.M. The one-way analysis of variances (ANOVA) followed by Dennett's multiple comparison test was used to analyze the data. We used the SPSS (version 21) and Graph-pad prism statistical software (version 6.1) for data analysis and figure creation. A significant difference was considered between the groups when $P<0.05$.

RESULTS

Serotonin scratching effect study

Serotonin, administered intradermally, elicited scratching of the injected site. Figure 1 shows that serotonin produced a significant enhancement in scratching (F (5, 32) =65.30, $P<0.0001$). This agent produced a dose-dependent enhancement in scratching from 14.1nmol/site to 141nmol/site in animals. The dose–response curves for serotonin was bell-shaped. The maximal pruritogenic effect of serotonin was observed at the dose 141 nmol/site in comparison to intradermally saline-injected animals ($P<0.0001$). Also, results have shown that intradermal saline injection did not affect the scratching behavior in mice compared to untreated control group ($P>0.05$).

Role of 5-HT3 receptors subtype in serotonin-induced scratching

Ondansetron and tropisetron (50, 100, and 200 nmol/site, i.d.) did not induce significant scratch behaviors comparing to saline injected animals (F (4, 35) =0.1772, P>0.05, data not shown) and (F (4, 35) =0.0942, P>0.05, data not shown), respectively.

Figure 2 shows that ondansetron and tropisetron notably diminished the scratching effect of intradermal serotonin. Ondansetron and tropisetron suppressed the scratching effect of intradermal serotonin ((F (4, 35) =34.17, P<0.001) and (F (4, 35) =8.868, P<0.001) respectively). Concurrent administration of ondansetron (50, 100, and 200 nmol/site, i.d.) (P<0.05, P<0.001, and P<0.001 respectively, Fig. 2A) and tropisetron (100 and 200 nmol/site, i.d.) (P<0.05 and P<0.001 respectively, Fig. 2B) with serotonin (141 nmol/site, i.d.) reduced scratching behavior comparing to the animals that received serotonin alone. Also, data have shown that intradermal saline administration did not affect the scratching behavior induced by serotonin in mice (P>0.05).

Ondansetron (0.1, 0.3, and 1 mg/kg, i.p.) and tropisetron (0.1, 0.3, and 1 mg/kg, i.p.) did not affect scratch behaviors of mice ((F (4, 35) =0.080, P>0.05, data not shown) and (F (4, 35) =0.1236, P>0.05, data not shown), respectively).

The effect of i.p. pretreatment of mice with 5-HT3 antagonists 30 minutes before intradermal serotonin (141 nmol/site) injection is depicted in Figure 3. Intraperitoneal ondansetron and tropisetron significantly suppressed scratching due to serotonin injection (F (4, 27) =9.490, P<0.001) and (F (4, 35) =6.348, P<0.01). Serotonin-induced scratching behavior was considerably diminished in mice that had received ondansetron (0.3 and 1 mg/kg, i.p) (P<0.01 and P<0.001 respectively, Fig. 3A) and tropisetron (0.3 and 1 mg/kg, i.p.) (P<0.05 and P<0.01 respectively, Fig. 3B) prior to serotonin, compared to saline-pretreated mice. However,

i.p. ondansetron and tropisetron (0.1 mg/kg) did not significantly abolish serotonin-evoked itch response compared to the saline-pretreated group (P>0.05). Data have shown that systemic saline administration did not alter the scratching behavior induced by serotonin in mice (P>0.05).

DISCUSSION

In this study, we measured the hind limb scratching directed toward a site of pruritic treatment as a method to examine itch response. In rodents, acute pruritogen-induced scratching behavior routinely manifests as multiple bouts of variable duration; each bout consists of rapid back-and forth movements of the toenails of the hind paw across the region of the treated site [6,21]. Herein, we showed the serotonin-induced scratching activity in mice. Intradermal administration of ondansetron and tropisetron attenuated the itch induced by intradermal serotonin. Also, i.p. pre-treatment with ondansetron and tropisetron, 30 minutes before intradermal serotonin injection, inhibited the scratching behavior, which was observed with the serotonin administration alone. Therefore, we concluded that the scratching effect of 5-HT might be partly mediated by its subtype 3 receptors.

Itch can be regarded as a physiological defense mechanism to counteract harmful external agents [2]. It is one of the most suffering sensations and can considerably impair the quality of life [22]. Itch can be originally peripheral (dermal, neuropathic) or central (neuropathic, neurogenic, psychogenic) [5]. Itching is a multifactorial condition involving multiple mediators at the cellular and molecular levels [23]. Different etiologies may underlie the phenomenon of itching. Recent reports have suggested four commonly regarded itching pathways, the TRPV1 channel, histamine, PAR2, and serotonin pathways [23].

Serotonin elicits an itch sensation when applied to rodent [6,21] and human skin [7] and has been suggested to be involved in the itching associated with pruritic diseases, such as cholestasis [9] and polycythemia vera [8]. The dose–response curve of 5-HT administration in mice is bell-shaped. In humans, experimentally-induced itch after intradermal 5-HT injection is less pruritic in comparison to histamine [24], although 5-HT may play a chief role in chronic pathological itch [13,17]. In contrast, mast cells of rodents majorly contain 5-HT rather than histamine [25] and therefore, intradermal 5-HT is a more potential scratch inducer in mice. However, the cellular mechanisms through that intra-dermal 5HT induces pruritus have not been fully investigated.

A few studies were conducted to evaluate the role of 5-HT receptor subtypes in serotonin-evoked itching. It was shown that serotonin-induced itch response was reduced by ketanserin, a 5-HT2 subtype antagonist, in rats [12]. Likewise, Yamaguchi et al stated that 5HT2 receptors are capable of inducing itch following intradermal serotonin injection [6); nonetheless, they demonstrated that per-oral pretreatment with ondansetron and MDL-72222, another 5-HT3 antagonist, did not significantly suppress 5-HT-induced itching in male ddY mice [6]. It is worth mentioning that experimentally serotonin-induced flares in human subjects was decreased by 5-HT3 antagonist, tropisetron, treatment [7,26]. On the other hand, association of 5-HT receptors subtype 3 in itching induced by some diseases has been shown in several studies. Administration of ondansetron and granisetron, 5-HT3 antagonists, obviously reduced the pruritus related to cholestasis [9,27]. In addition, ondansetron administration relieved symptoms of itch in uremic patients [14,17]. Also, ondansetron relieved the opioid-induced itch in humans [16,28]. The effectiveness of ondansetron on this type of pruritus leads to the suggestion that serotonin, acting via 5-HT3 receptors, is involved in generation and sensation of itch. These receptors are existing

on neurons in the autonomic and the central nervous systems [29]. The pain-producing and pain-enhancing effects of serotonin are abolished by 5-HT3 receptors antagonists [30,31]. It can be speculated that itching may be attenuated by blockade of 5-HT3 receptors on sensory nerve endings. In our current experiment, for the first time, serotonin-induced scratching was partly suppressed by systemic as well as intradermal injection of ondansetron and tropisetron, suggesting that peripheral 5-HT3 receptor may be involved in serotonin-induced scratching in mice. In a previous study, Yamaguchi et al concluded that 5-HT3 antagonist, ondansetron, did not centrally suppress scratching due to serotonin in mice [6]. The most probable explanation for their different observation from ours is basically the time and route of ondansetron administration in that study. They pretreated the mice with ondansetron per-orally in order to investigate its central effect on pruritus; however it is worth mention that the bioavailability of oral ondansetron might be too low in mice, since this fact has been demonstrated in rats [32,33], and therefore could be plausible in other rodents. Especially when using low doses, such as those used in the aforementioned study, the high systemic clearance due to first-pass metabolism results in a short half-life and low systemic bioavailability [32,33]. Therefore, they should not have expected to notice a significant antipruritic effect 1 hour after per oral administration of 0.01-1 mg/kg ondansetron. Plus, we have used different strains of mice and that might also be another cause of dissimilarity of our outcomes with ondansetron treatment on serotonin-induced itch. However, those doses of ondansetron had certainly elicited antipruritic effect, but owing to the high degree of error, the result was not considered significantly different from the control group.

CONCLUSIONS

In summary, we have for the first time proposed peripheral antipruritic effect of ondansetron and tropisetron in mice. Thus, it can be concluded that 5-HT3 antagonists represent a new class of specific receptors antagonist suitable for treatment of pruritus evoked by serotonin.

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Figures:

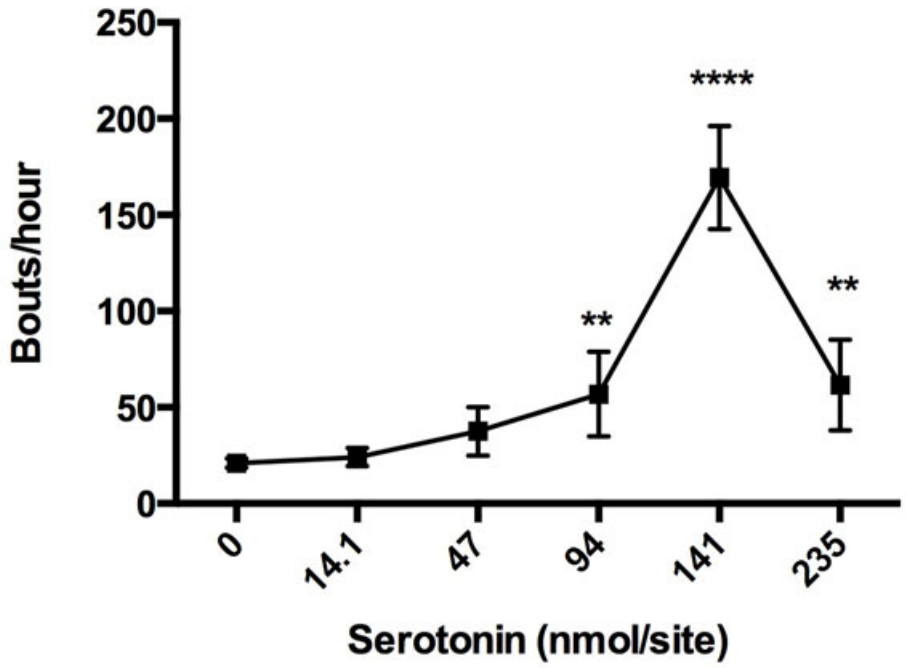

Figure 1

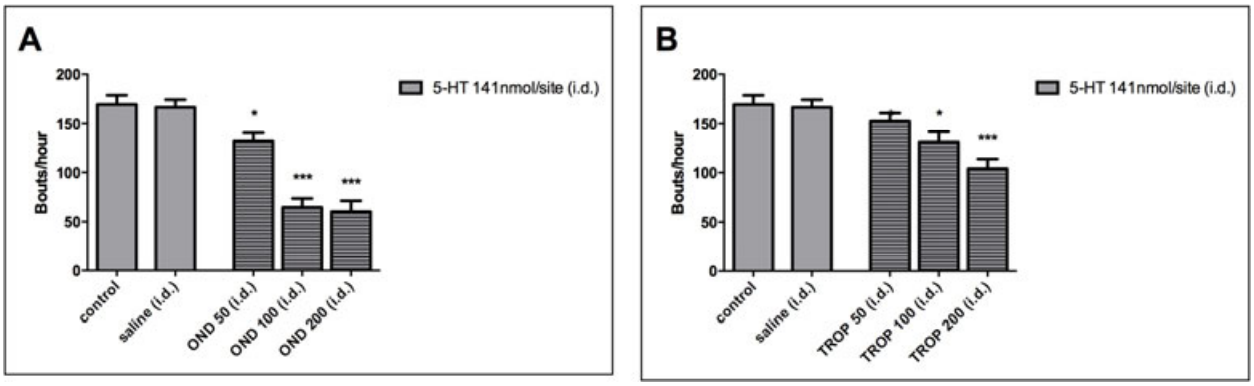

Figure 2

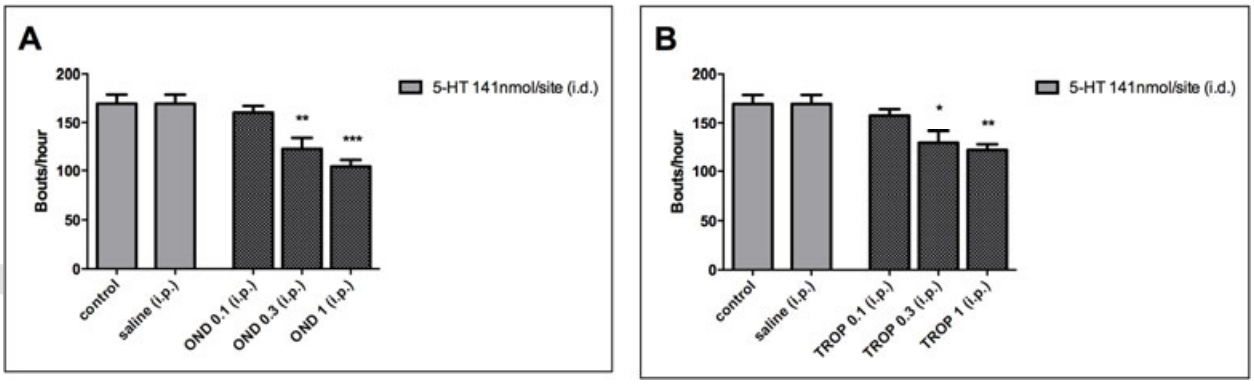

Figure 3

FIGURE LEGENDS

Figure 1. The dose-response curve for intradermal injection of serotonin in mice. Mice were given an intradermal injection of saline or serotonin at doses of 14.1, 47, 94, 141 and 235 nmol/ site into the rostral back. The scratching bouts directed towards the injection site were counted for 60 min. Values represent the means ± S.E.M. of 6-8 animals, compared with saline-injected group, ** $P<0.01$ and **** $P<0.0001$.

Figure 2. Effect of different intradermal doses (50, 100, and 200 nmol/site) of (A) ondansetron and (B) tropisetron on scratching behavior in mice. Ondansetron and tropisetron were intradermally injected and scratching of the injected site was counted for 60 min. Values represent the means ± S.E.M. of 6-9 animals, compared with saline-injected group, * $P<0.05$ and *** $P<0.001$.

Figure 3. Effect of different intraperitoneal doses (0.1, 0.3, and 1 mg/kg) of (A) ondansetron and (B) tropisetron on scratching behavior in mice. Ondansetron and tropisetron were intraperitoneally injected and scratching of the injected site was counted for 60 min. Values represent the means ± S.E.M. of 6-8 animals, compared with saline-injected group, *$P<0.05$, ** $P<0.01$, and *** $P<0.001$.