Serotonin-induced Biting of the Hind Paw is Itch-related Response in Mice

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Abstract

Recently we have found that an injection of serotonin (5-HT) into the murine rostral back elicits scratching, a finding suggesting that serotonin is pruritogenic in the mouse. To elucidate the itch-related responses of the hind paw, we examined the behavioral responses to 5-HT injected into mouse hind paw and compared it with the responses to formalin, an algesciogenic agent. We examined also the effects of 5-HT on saphenous nerve activity of the mouse. An injection of saline into the hind paw produced licking, but not biting. 5-HT (30~100 nmol/site) clearly elicited biting, but licking was not significantly different between saline and 5-HT. Dilute formalin elicited marked licking and slightly biting. 5-HT (60 nmol/site)-induced biting was decreased by methysergide (10 nmol/site, injected together with 5-HT), a 5-HT receptor antagonist, and by the opioid antagonist naloxone (1 mg/kg, s.c.). In anesthetized mice, 5-HT (30~100 nmol/site) increased the activity of either intact or deafferented saphenous nerve. Licking and saphenous nerve activity were less after 5-HT (300 nmol/site) than that after the lower 5-HT dose (100 nmol/site), suggesting the multiple actions of 5-HT on the primary afferents. The present results suggest that biting of the injected site provoked by 5-HT administered into the murine hind paw is itch-related, but not pain-related, response.

Key words: itch-related response, biting and licking, serotonin (5-HT), mouse, saphenous nerve activity

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is

one of inflammatory mediators that are released in injured and inflamed tissue. It enhances pain produced by other inflammatory mediators in

humans and animals, but needs high doses to produce pain by itself^{2,17,18}. In addition, 5-HT elicits an itch sensation in humans^{14,21}. Although 5-HT itself elicits weak itching after intradermal injection, it produces a strong itch sensation when injected together with prostaglandins, which are also weak pruritogens by themselves¹⁴. 5-HT receptor antagonists were reported to inhibit pruritus in polycythaemia vera¹² and relieve cholestatic pruritus²³.

Itch and pain are considered to be different sensory modalities in humans. But it is difficult to discriminate between itch and pain responses of animals. Licking and lifting behaviors that are elicited by a subcutaneous (s.c.) injection of formalin into the hind paw have been regarded as pain responses^{1,2,10,26}. Scratching behavior which is elicited by intradermal injection of substance P into the rostral back has been considered to be itch-associated response^{3,4,19,27}. In many reports using 5-HT and formalin as painful agents, biting of treated site have been also regarded as pain responses^{1,2,10,26}. However, when injected into the rostral back of the mouse, 5-HT²⁷, but not formalin¹⁹, elicits scratching of the treated site by the hind paws, suggesting 5-HT produces itch rather than pain sensation in mice. The present experiments were conducted to examine whether itch and pain responses could be behaviorally distinguished. For this purpose, we examined the behavioral responses, especially biting and licking responses, to 5-HT injected s.c. into the hind paw, and compared the behavioral effects with the electrophysiological effects on the activity of saphenous nerve.

MATERIALS AND METHODS

Animals

Male ddY mice (7~9 weeks old) were used. The animals were housed under controlled temperature (23~25 °C) and light (lights on from 08:00 to 20:00). Food and water freely available.

Drugs

Serotonin hydrochloride (Sigma, St. Louis, USA), naloxone hydrochloride (Sigma) and methysergide malate (RBI, Massachusetts, USA)

were dissolved in physiological saline, and formalin (Wako Pure Chemical Ind., Osaka) was diluted with physiological saline. 5-HT and formalin were injected s.c. into the medial malleolus region of the hind paw in a volume of 20 μ l, and methysergide was injected together with 5-HT. Naloxone was injected s.c. into the rostral back 15 min before 5-HT injection.

Behavioral experiments

Before the experiment, each mouse was put into a clear plastic cage for about 30 min for acclimation. Immediately after the injection of 5-HT or formalin, the animal was put back into the same cage for the observation of biting and licking. The time of the behaviors was separately recorded on a pen recorder (Recti-Horiz 8 K, NEC San-ei, Tokyo) and the duration of the responses was calculated at 3-min intervals for 60 min.

Electrophysiological experiments

The mice were anesthetized with intraperitoneal injection of urethane (1.5 g/kg). They were lying on the back with the right ankle fixed on the board with adhesive tape. The skin of the medial region of the thigh was incised, and then the saphenous nerve was exposed and dissected free from the surrounding tissues. In experiments using the deafferented nerve, the saphenous nerve was cut at the proximal site. The nerve and blood vessels were immersed in mineral oil to prevent drying. The activity of the saphenous nerve was recorded extracellularly using bipolar electrode of silver wire and an AC bioelectric amplifier (AB651, Nihon Kohden, Tokyo) with band-pass of 1 kHz. Action potentials with the amplitude more than 40 μ V were adopted. The number of action potentials was analyzed at 3-min intervals from 15 min before to 60 min after the 5-HT injection using a histogram analyzer (QC-111J, Nihon Kohden).

Statistics

Statistical comparisons were done using one-way analysis of variance and post hoc Dunn's test; $p < 0.05$ was considered as significant.

Fig. 1 Time course of biting and licking responses after injection of 5-HT and formalin into the mouse hind paw. The mice were given an s.c. injection of saline (**a**), or 5-HT at doses of 30 (**b**), 60 (**c**), 100 (**d**) and 300 nmol/site (**e**), or 3% formalin (**f**). The response time of biting (●) and licking (○) per 3 min was plotted against time after the injection. Values are means $\pm$ s.e.m. of 6–7 mice.

RESULTS

Biting and licking responses induced by 5-HT and formalin

We observed separately biting and licking responses evoked by an s.c. injection of 5-HT or formalin into the mouse hind paw. An injection of saline elicited licking, but not biting (**Fig. 1a**). 5-HT at a dose of 30 nmol/site elicited licking and slight biting; the degree of licking was similar to that after saline (**Fig. 1b**). 5-HT at doses of 60 and 100 nmol/site substantially elicited biting as well as licking; the biting appeared 3–10 min after injection and subsided by 50 min (**Fig. 1c and 1d**). After a higher dose of 5-HT (300 nmol/site), both biting and licking responses were markedly decreased (**Fig. 1e**). In

Fig. 2 Effects of naloxone (NLX) and methysergide (MSG) on biting and licking elicited by 5-HT. 5-HT (60 nmol/site) was injected s.c. into the mouse hind paw. MSG (10 nmol/site) was injected together with 5-HT, and NLX (1 mg/kg) was injected s.c. into the rostral back 15 min before 5-HT. The response time of licking (open column) and biting (hatched column) for 60 min after 5-HT injection is shown. Values are means $\pm$ s.e.m. of 6–7 mice. * $p < 0.01$ when compared with 5-HT alone.

contrast to 5-HT, formalin injection elicited marked licking and only slight biting; licking was apparent immediately after the injection, rapidly reduced at 4–15 min and reappeared from 15 to 45 min (**Fig. 1f**).

Inhibition of 5-HT-induced biting by methysergide and naloxone

Pretreatment with the opioid antagonist naloxone (1 mg/kg, s.c.) significantly ($p < 0.01$) inhibited biting, but not licking, induced by 5-HT (60 nmol/site) (**Fig. 2**). When injected together with 5-HT (60 nmol/site), methysergide (10 nmol/site), a 5-HT receptor antagonist⁽⁸⁾, produced a marked and significant ($p < 0.01$) inhibition in the 5-HT-induced biting, without effect on the licking (**Fig. 2**).

Effects of 5-HT on the saphenous nerve activity

To examine the action of 5-HT at biting-inducing doses on primary afferents, we investigated the effects of 5-HT on the activity of

Fig. 3 Time course of the effect of 5-HT on the activity of intact saphenous nerve. The mice were given an injection of saline (a) or 5-HT at doses of 30 (b), 60 (c), 100 (d) and 300 nmol/site (e) into the hind paw. The number of action potentials per 3 min was plotted against time. Values are means $\pm$ s.e.m. of 5~6 mice.

intact and deafferented saphenous nerve. In the intact nerve, saline injection produced slight increase of firing, and 5-HT at doses of 30~100 nmol/site produced a dose-dependent increase in the firing, which was apparent within a few minutes and lasted for 15~45 min depending on the dose examined (**Fig. 3**). The effect of 5-HT at a dose of 300 nmol/site was as small as that of the lowest dose examined (30 nmol/site). In the deafferented nerve, although saline and 5-HT at a dose of 30 nmol/site produced slight increase of firing, 5-HT at doses of 60 and 100 nmol/site produced marked increase of firing in a dose-dependent manner (**Fig. 4**). 5-HT at a dose of 300 nmol/site substantially induced firing, but the effect was smaller than that of a lower dose of 100 nmol/site (**Fig. 4**). **Figure 5** compares the dose-dependency of the behavioral and electrophysiological effects of 5-HT. Regardless of whether the nerve was intact or deafferented, the dose-response relationship of the action of 5-HT

Fig. 4 Time course of the effect of 5-HT on the activity of deafferented saphenous nerve. The mice were given an injection of saline (a) or 5-HT at doses of 30 (b), 60 (c), 100 (d) and 300 nmol/site (e). The number of action potentials per 3 min was plotted against time. Values are means $\pm$ s.e.m. of 5~7 mice.

on the discharge of the saphenous nerve with the amplitude more than 40 μ V corresponded to the biting-inducing action of 5-HT.

DISCUSSION

One important finding in the present experiments is that an injection of 5-HT into the hind paw elicited biting. The biting-inducing doses (30~100 nmol/site) of 5-HT (present experiments) correspond to the scratching-inducing doses of 5-HT after injection into the rostral back²⁷⁾. These results raise the possibility that 5-HT-induced biting is itch-associated response. To ascertain this possibility, we examined the effect of opioid antagonist on the behavioral actions of 5-HT. Systemic pretreatment with naloxone produced marked and significant inhibition of 5-HT-induced biting, without effect on the licking. Naloxone suppresses histamine-induced itch in healthy subjects²⁹⁾. It also ameliorates itch sensation of patients with pruritic

Fig. 5 Dose-response curves for the behavioral and electrophysiological effects of 5-HT injected into the mouse hind paw. (a) Biting and licking behaviors after 5-HT injection. The response time of biting (●) and licking (○) for 60 min after 5-HT injection was plotted against the dose. $n=6\sim7$. (b) Firing of the saphenous nerve after 5-HT injection. The total number of action potentials of intact (●) and deafferented (▲) saphenous nerves for 60 min after 5-HT injection was plotted against the dose. $n=5\sim7$. Values are means $\pm$ s.e.m. * $p<0.05$ when compared with saline.

diseases, especially cholestasis, and inhibits scratching of the patients⁶⁾. In animals, naloxone inhibits scratching induced by substance P⁴⁾ and 5-HT²⁷⁾. On the other hand, naloxone increases, rather than inhibits, behavioral pain responses^{16,24)}. Taking account of these findings, our results suggest that 5-HT-induced biting is due to an itch sensation of the treated skin. Biting

may be a painful counterstimulus against an itch sensation²²⁾.

5-HT-induced biting was markedly suppressed by co-injection with the 5-HT receptor antagonist methysergide, finding suggesting that the biting response is elicited via an activation of peripheral serotonergic receptors. Although it remains unknown which subtype(s) of serotonergic receptors are involved in the 5-HT action, in our preliminary experiments, the 5-HT₂ receptor agonist α -methylserotonin (100 nmol/site) apparently elicited biting, suggesting the involvement of 5-HT₂ receptors.

Painful stimulation with formalin elicited markedly licking and slightly biting. The result is consistent with other reports using mice, rats and cats^{1,2,10,24,26)}. Although 5-HT injection elicited licking also, the duration of licking response was not significantly different between 5-HT and saline. Therefore, licking after 5-HT injection might be primarily due to pain induced by the injection manipulation. This view is also supported by the finding that 5-HT-induced licking was not inhibited by methysergide.

Peripheral injection of 5-HT activates nociceptive fibers^{5,13)}. It has been hypothesized that 5-HT has a direct action on primary afferents²⁵⁾. Pharmacological studies indicate that 5-HT_{1A}, 5-HT_{2A} and/or 5-HT₃ receptors are involved in acute inflammation and/or peripheral hyperalgesia^{2,11,15)} and thus are likely to be present in the primary afferents. Furthermore, an immunohistochemical study has proved the presence of 5-HT_{2A} receptors on primary afferents at the dermal-epidermal junction in rat glabrous skin⁹⁾. In our present study, we showed that 5-HT injected into medial malleolus region, which is one of the receptive fields of saphenous nerve, of mouse hind paw increased the number of action potentials in a dose-dependent manner. The time courses of stimulating effects of 5-HT on the intact afferents were similar to that in the deafferented fibers. 5-HT enhanced the total number of action potentials of both the intact and deafferented fibers in the same dose range as provoking apparent biting response in conscious mice. At the highest dose of 300 nmol/site, 5-HT reduced rather than augmented the total number

of the action potentials not only in the intact fibers but also in the deafferented fibers, as compared with the effect of a dose of 100 nmol/site. These results suggest that the 5-HT-induced bell-shaped effect on saphenous nerve activity resulted from an activation of 5-HT receptors on the primary afferents in the skin. As it has been proposed that some subtypes of 5-HT receptor exist in primary afferents^{2,9,11,15}, the simultaneous activation of receptor subtypes may produce the bell-shaped effect of 5-HT. Other inflammatory mediators provided by 5-HT-induced extravasation may be also involved in the bell-shaped effect, although the precise mechanisms of the 5-HT effect on the primary afferents remain to be settled.

Systemic pretreatment with naloxone did not affect 5-HT-induced licking. In contrast, naloxone has been shown to increase formalin-induced licking^{16,24}. The apparent discrepancy between 5-HT- and formalin-induced licking could be explained by differences in the magnitude and duration of pain. Formalin treatment gradually increases the release of Met-enkephalin from the nucleus reticularis gigantocellularis of the medulla oblongata²⁰. Naloxone increases formalin-induced licking from 30 to 60 min after formalin injection, without effects on licking during initial 30 min²⁴. On the other hand, as discussed above, licking after 5-HT injection may be due to pain induced by injecting manipulation rather than 5-HT.

In conclusion, 5-HT injection into the hind paw elicited biting of the injected site. This response may be due to an itch sensation rather than pain because it was inhibited by naloxone. The 5-HT-induced biting is an experimental itch model useful for studying the itch-signaling mechanisms in the spinal cord.

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