

## Effects of histamine on spontaneous neuropathic pain induced by peripheral axotomy

Jie Yu<sup>1#</sup>, Guo-Dong Lou<sup>1#</sup>, Jia-Xing Yue<sup>1</sup>, Ying-Ying Tang<sup>2</sup>, Wei-Wei Hou<sup>1</sup>, Wen-Ting Shou<sup>1</sup>, Hiroshi Ohtsu<sup>3</sup>, Shi-Hong Zhang<sup>1</sup>, Zhong Chen<sup>1</sup>

<sup>1</sup>*Department of Pharmacology, School of Basic Medical Sciences; College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China*

<sup>2</sup>*School of Pharmaceutical Sciences, Wenzhou Medical College, Wenzhou 325035, China*

<sup>3</sup>*Department of Engineering, School of Medicine, Tohoku University, Aoba-ku, Sendai 980-8775, Japan*

#These authors contributed equally to this work.

Corresponding authors: Zhong Chen and Shi-Hong Zhang. E-mail: [chenzhong@zju.edu.cn](mailto:chenzhong@zju.edu.cn); [shzhang713@zju.edu.cn](mailto:shzhang713@zju.edu.cn)

© Shanghai Institutes for Biological Sciences, CAS and Springer-Verlag Berlin Heidelberg 2013

### ABSTRACT

The present study was designed to investigate the effects of histamine on spontaneous neuropathic pain (NP) induced by peripheral axotomy. Rats and mice were subjected to complete transection of the left sciatic and saphenous nerves to induce spontaneous NP (the neuroma model). Rats were then treated with drugs once daily for 30 days (histidine and loratadine, i.p.) or 21 days (histamine, i.c.v.). Autotomy behavior was scored daily until day 50 post-operation (PO). On days 14 to 21 PO, some rats in the control group were subjected to single-fiber recording. Autotomy behavior was also monitored daily in histidine decarboxylase (the key enzyme for histamine synthesis) knockout (HDC<sup>-/-</sup>) and wild-type mice for 42 days. We found that both histidine (500 mg/kg) (a precursor of histamine that increases histamine levels in the tissues) and histamine (50 µg/5 µL) significantly suppressed autotomy behavior in rats. HDC<sup>-/-</sup> mice lacking endogenous histamine showed higher levels of autotomy than the wild-type. In addition, the analgesic effect of histidine was not antagonized by loratadine (a peripherally-acting H<sub>1</sub> receptor antagonist), while loratadine alone significantly suppressed autotomy. Electrophysiological recording showed that ectopic spontaneous discharges from the neuroma

were blocked by systemic diphenhydramine (an H<sub>1</sub> receptor antagonist). Our results suggest that histamine plays an important role in spontaneous NP. It is likely that histamine in the central nervous system is analgesic, while in the periphery, *via* H<sub>1</sub> receptors, it is algescic. This study justifies the avoidance of a histamine-rich diet and the use of peripherally-acting H<sub>1</sub> receptor antagonists as well as agents that improve histamine action in the central nervous system in patients with spontaneous NP.

**Keywords:** histamine; loratadine; H<sub>1</sub> receptor; neuropathic pain

### INTRODUCTION

Neuropathic pain (NP) is defined as pain initiated or caused by a primary lesion or dysfunction of the nervous system. It occurs in many common diseases, following injuries and in response to drugs or toxins. The pain may be spontaneous, stimulus-evoked, or a combination of both, and spontaneous pain is a predominant complaint. Although it afflicts ~1% of the population<sup>[1,2]</sup>, NP remains a challenge. Classical analgesics such as opioids and NSAIDs are usually inadequate. Some antidepressants and antiepileptic drugs are partially effective but often have treatment-limiting adverse effects<sup>[1,3]</sup>. Therefore, further investigation of NP

pathophysiology is essential for developing approaches to achieve efficient analgesia.

Histamine acts as a neurotransmitter to regulate a variety of functions, including food intake, body temperature, memory retention, wakefulness and pain sensation. In acute pain sensation, the histamine effect can either be analgesic or algescic, depending on the site of action and the dose<sup>[4–6]</sup>. As to NP, however, most reports focus on the effect of histamine on evoked NP and the results are controversial. For instance, local injection of H<sub>1</sub> receptor antagonists attenuates the pain abnormalities induced by partial nerve injury<sup>[7]</sup>, while histamine infused directly into the brain is either algescic or analgesic on evoked NP depending on the dose<sup>[8]</sup>. Some H<sub>3</sub> or H<sub>4</sub> receptor antagonists inhibit the evoked NP in several animal models<sup>[9,10]</sup>, but others have no effect<sup>[10]</sup>. In contrast, very few studies have addressed the effect of histamine on spontaneous NP. Baron *et al.* found that cutaneous histamine application induces spontaneous pain in neuropathic skin<sup>[11]</sup>, while Suzuki *et al.* reported that the H<sub>1</sub> antagonist mepyramine does not affect the ongoing firing of deep dorsal horn neurons induced by spinal nerve ligation<sup>[12]</sup>. These studies imply an intriguing relationship of histamine with spontaneous NP, which deserves further investigation.

The neuroma model in rodents is produced by total denervation of one hindpaw<sup>[13]</sup>. Animals subjected to the neuroma model show a series of pain-like behaviors termed autotomy. Because its temporal and spatial parameters can be quantified, autotomy has been widely used to describe the onset, duration and level of spontaneous pain<sup>[14,15]</sup>. Therefore, in the present study, we used the neuroma model to investigate the effects of histamine on spontaneous NP.

## MATERIALS AND METHODS

### Animals

Eighty-three male adult Sprague-Dawley rats (260–300 g, Grade II, Certificate No. SCXK2003-0001, Experimental Animal Center, Zhejiang Academy of Medical Science, Hangzhou, China), 11 histidine decarboxylase knockout (HDC<sup>-/-</sup>) mice (kindly provided by Professor Ohtsu<sup>[16]</sup>) and 11 of their wild-type littermates (HDC<sup>+/+</sup>) were used in this study. Animals were raised at 22 ± 0.5°C under a 12:12 h light/dark cycle (lights on from 08:00 to 20:00) with food

and water *ad libitum*. All experiments were in accordance with the guidelines of the International Association for the Study of Pain<sup>[17]</sup> and approved by the Animal Care and Use Committee of Zhejiang University. Efforts were made to minimize the number of animals used and their suffering.

### The Neuroma Model and Autotomy Follow-Up

The neuroma model was induced as described previously<sup>[18,19]</sup>. Briefly, rats and mice were deeply anaesthetized by inhalation of isoflurane. The sciatic nerve was exposed and isolated by blunt dissection and tightly ligated with 5-0 silk just proximal to its trifurcation. Another ligation was made 2 mm distal to the first and then the nerve was cut between the two ligatures. About 2–3 mm of the tibial, sural and common peroneal branches were excised to prevent nerve regeneration. The saphenous nerve was then exposed medial to the knee and transected. A 3-mm segment was removed. The wounds were then closed in layers.

Animals were housed individually to prevent social interactions from affecting autotomy behavior<sup>[20]</sup>. Levels of autotomy were scored daily postoperatively (PO), from the day of surgery until day 50 PO for rats and day 42 PO for mice, using an accepted scale. In this scale, one point was assigned for removal of at least two nails, and one additional point for every ½ toe injured, to a maximum of 11<sup>[18]</sup>. When animals showed autotomy with 11 points, they were euthanized and their scores were retained for the remainder of the behavioral follow-up until the last day of observation.

### Single-fiber Recording

At days 14 to 21 PO, rats were deeply anaesthetized with urethane (1.2 g/kg) and the right jugular vein was cannulated to enable systemic injection of diphenhydramine or saline. Rats were placed on a small heating blanket that was feedback-controlled by a rectal thermometer, thereby maintaining a core temperature of 37°C. Rats were paralyzed with gallamine triethiodide (10 mg/kg initially, supplemented when needed) and artificially ventilated throughout the experiment. The injured sciatic nerve was re-exposed and a pool constructed by tying flaps of thigh skin to a frame was filled with warm paraffin oil (34°C) to protect the nerve from drying.

Under a microscope, the perineurium was opened carefully and microfilaments were teased from the nerve trunk with a pair of sharply-honed jewelry forceps. The mi-

crofilaments were placed on a pair of Ag/AgCl electrodes that were referenced to a nearby muscle. These filaments contained a few axons, usually 3–4, of which no more than 2 fired spontaneously. Signals were fed to an amplifier (Cyberkinetics, USA) and the digitized data were then analyzed offline by dedicated software (Neuroexplorer, USA). When a microfilament was identified as having a unit with spontaneous firing, at least 10 min of baseline firing data were collected before administration of diphenhydramine or saline.

### Drug Administration

Rats were weighed and postoperatively given histidine (200 or 500 mg/kg), loratadine (10 mg/kg), or their vehicles (40% propylene glycol or 60% saline, 1 mL) once daily (between 09:00 and 11:00) *via* intraperitoneal injection (i.p.) through days 0–30 PO. For intracerebroventricular (i.c.v.) microinjection of histamine, a stainless-steel cannula (Reward, China) was implanted into the left lateral cerebral ventricle (AP: –0.96 mm, L: –2.0 mm, V: –4.0 mm) and then embedded in the skull with dental cement seven days before nerve injury. Histamine (50 µg) or saline, both in 5 µL, was injected once daily in 10 min through days 0–21 PO *via* a disposable dental needle (30 G; Nipro Medical Industries Ltd, Japan), which was attached to a 15–20 cm PE-10 tube fitted to a 10 µL Hamilton syringe. In the single-fiber recordings, a bolus of diphenhydramine (25 mg/kg per bolus of 0.1 mL) or saline was injected through the cannulated jugular vein in one minute.

### Data Analysis

The postoperative day when the animal started autotomy behavior was defined as the onset day and was analyzed by a nonparametric test (Mann-Whitney test). Values of the area under the curve (AUC) were analyzed by one-way ANOVA followed by Dunnett's test. Statistical analysis was performed with SPSS (ver. 16.0) for Windows and  $P < 0.05$  was considered statistically significant.

## RESULTS

### Postoperative Systemic Administration of Histidine Attenuated Autotomy in Rats

Compared with the control group (vehicle;  $n = 8$ ), postoperative histidine (200 mg/kg,  $n = 9$ ) significantly delayed the

onset of autotomy ( $6.43 \pm 2.20$  vs  $13.43 \pm 2.21$  days PO,  $P < 0.05$ ), but did not yield a remarkable reduction in the AUC during the period of administration (days 0–30 PO) or during the observation period after the cessation of drug treatment (days 31–50 PO) (Fig. 1). However, the progression of autotomy was suppressed by histidine at 500 mg/kg: the onset of autotomy was postponed to  $18.00 \pm 4.68$  days PO ( $n = 8$ ;  $P < 0.05$  vs vehicle-treatment group; Fig. 1A, B); and the mean autotomy scores were reduced, as manifested by the decreased AUC of autotomy progression, during the period of administration ( $P < 0.05$ ) and after the cessation of drug treatment ( $P < 0.001$ ; Fig. 1C, D).

### Postoperative Central Administration of Histamine Attenuated Autotomy in Rats

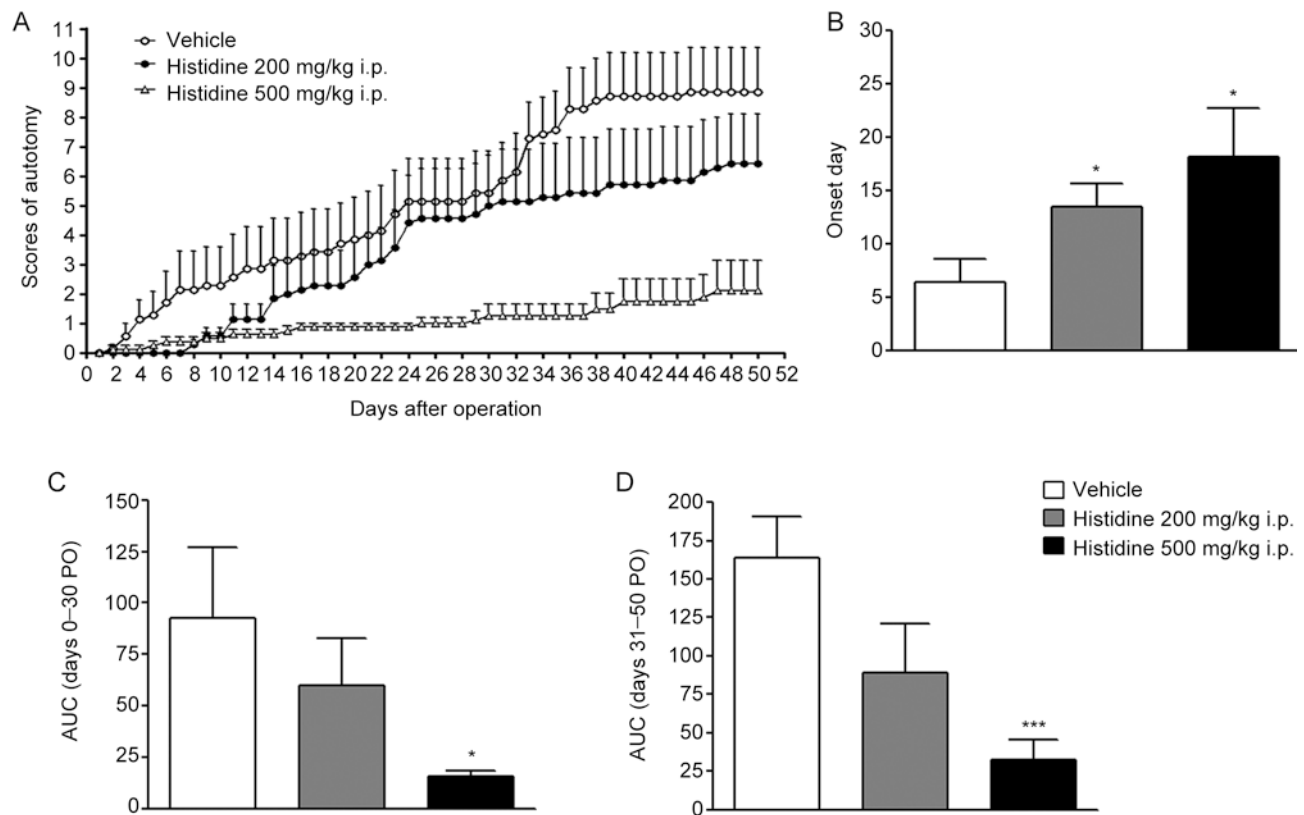
Intracerebroventricular injection of histamine (50 µg) significantly suppressed the autotomy behavior (Fig. 2A). The onset day was postponed to  $29.00 \pm 7.60$  ( $n = 6$ ), compared to the saline group ( $6.17 \pm 1.45$ ,  $n = 6$ ,  $P < 0.05$ ; Fig. 2B). The AUC during the period of histamine administration (days 0–21 PO) and during the observation period after the cessation of drug treatment (days 22–50 PO) were both lower than that in the saline-treated group ( $P < 0.05$  and  $0.001$ , respectively; Fig. 2C, D).

### HDC<sup>-/-</sup> Mice Showed Higher Levels of Autotomy than the Wild-Type

After total denervation of one hindpaw, HDC<sup>-/-</sup> mice showed significantly higher autotomy scores than HDC<sup>+/+</sup> mice ( $n = 11$ /group, Fig. 3A). Although no difference was detected between the two genotypes in the onset day of autotomy ( $P > 0.05$ ; Fig. 3B), the AUC in HDC<sup>-/-</sup> mice during the observation period was remarkably higher than that in HDC<sup>+/+</sup> mice ( $106.60 \pm 28.64$  vs  $30.94 \pm 5.79$ ;  $P < 0.05$ ; Fig. 3C).

### Loratadine Co-Administered with Histidine or Administered Alone Attenuated Autotomy in Rats

In order to investigate the mechanisms underlying the analgesic effect of histidine, loratadine at a commonly-used dose (10 mg/kg) was co-administered with histidine (500 mg/kg). Loratadine did not antagonize the inhibition of autotomy by histidine, but significantly suppressed autotomy (Fig. 4A). The onset day was delayed to  $28.50 \pm 6.19$  ( $n = 8$ ,  $P < 0.01$ ) and the progression was remarkably inhibited, demonstrated by the significant reduction of the AUC during



**Fig. 1.** Effect of histidine on autotomy behavior in rats. Animals were intraperitoneally injected with histidine at 200 or 500 mg/kg. **A:** Progression of autotomy. **B:** Onset day of autotomy. **C** and **D:** The area under the curve (AUC) for days 0–30 and 31–50 postoperatively (PO). \* $P < 0.05$ , \*\*\* $P < 0.001$  vs vehicle control group.

and after the drug treatment compared with that in the vehicle-treated group ( $P < 0.01$  and  $0.001$ , respectively; Fig. 4C and D). In addition, loratadine administered alone delayed the onset day to  $24.60 \pm 6.43$  ( $n = 10$ ,  $P < 0.05$ ) and inhibited the progression of autotomy ( $P < 0.01$  and  $0.001$ , respectively; Fig. 4).

#### Diphenhydramine Blocked Spontaneous Firing from the Neuroma

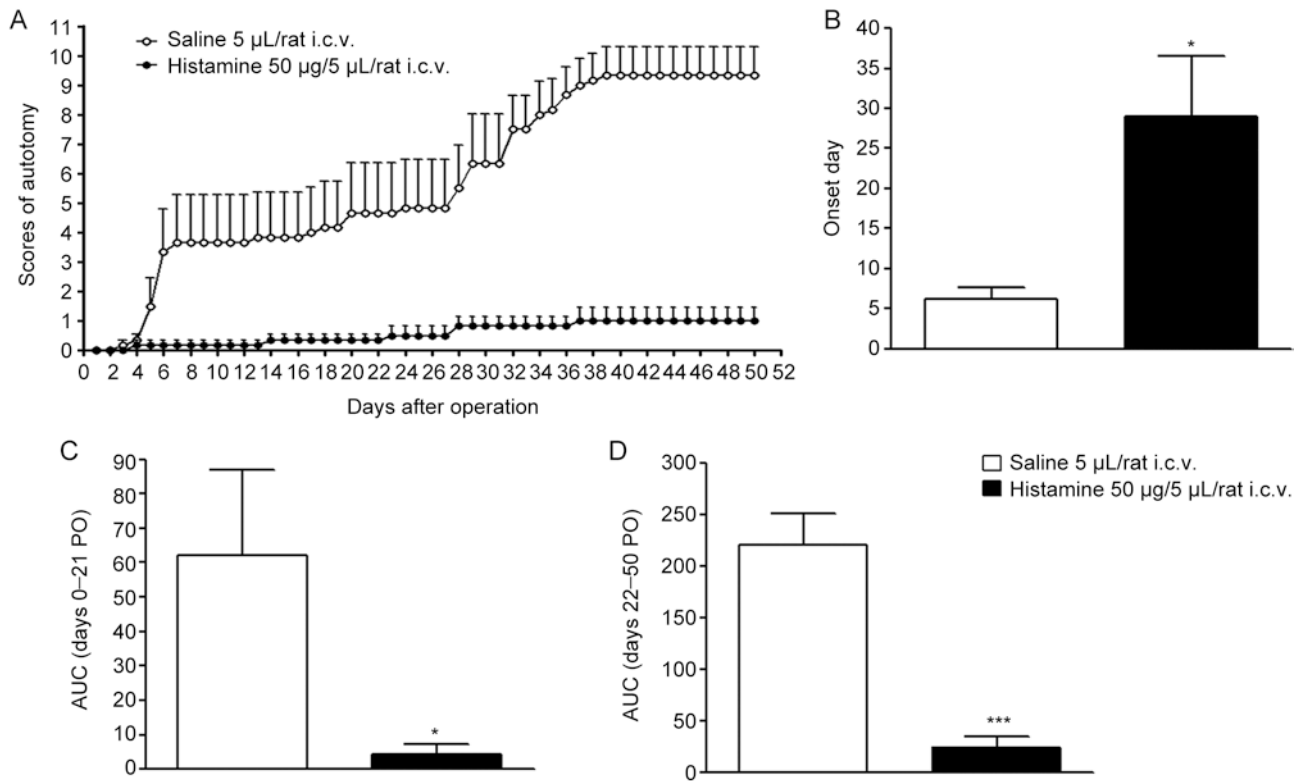
At days 14–21 PO, the majority of nerve filaments in the sciatic nerve contained units with ongoing spontaneous activity. The firing pattern varied from an irregular discharge of single impulses, present in about 70%, to bursts of several impulses. If the firing ceased for at least 60 s after drug administration, it was considered to be blocked. Fig. 5 shows that two boluses of diphenhydramine (25 mg/kg) resulted in a complete and reversible blockade of ongoing discharge in a fiber, lasting ~90 min. However, injection of

an equivalent volume of saline had no such effect. In trials where diphenhydramine blocked the spontaneous firing, latency to the blockade was usually less than 10 s, at most 30 s. The  $ED_{50}$  for blockade was 50.92 mg/kg ( $n = 9$ ) and the averaged blocking duration was >30 min.

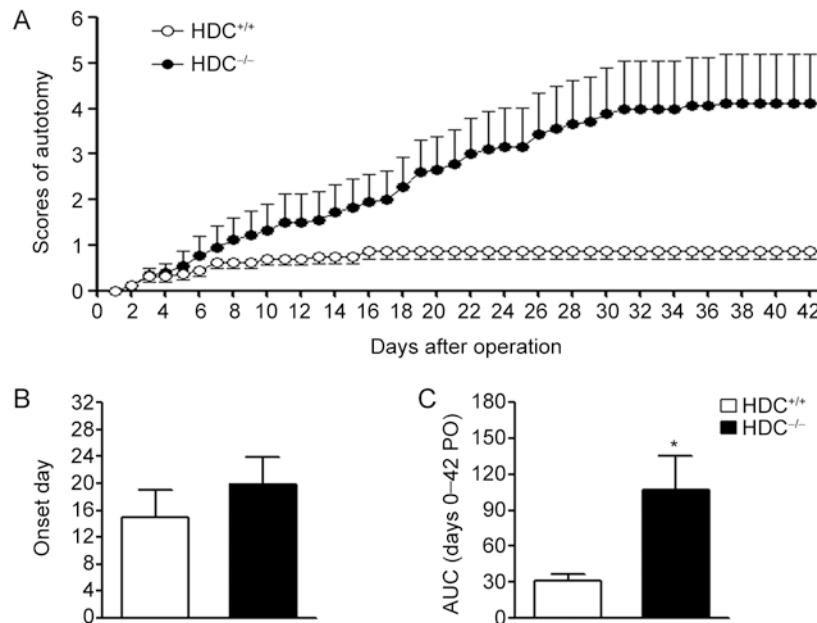
#### DISCUSSION

In the present study, we found that both histidine, the precursor of histamine, and histamine suppressed autotomy behavior in the neuroma model. Moreover, mice lacking endogenous histamine showed higher levels of autotomy than their wild-type littermates. These results indicate that histamine plays important roles in spontaneous NP, and may be a potential target for the treatment of this disorder.

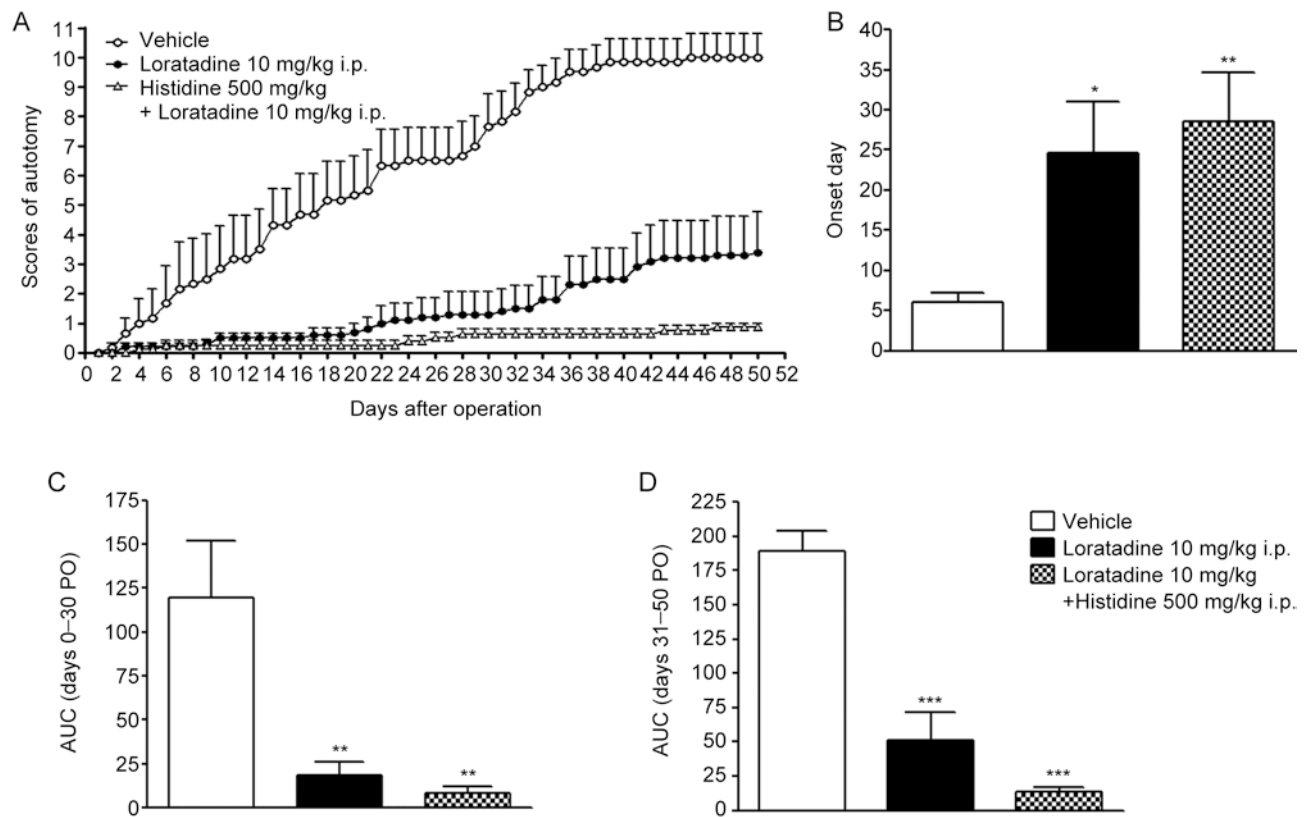
As shown in Fig. 1, after total denervation of one hindpaw, rats showed autotomy to different extents as reported previously<sup>[18,19]</sup>. In order to evaluate the effect of histamine,



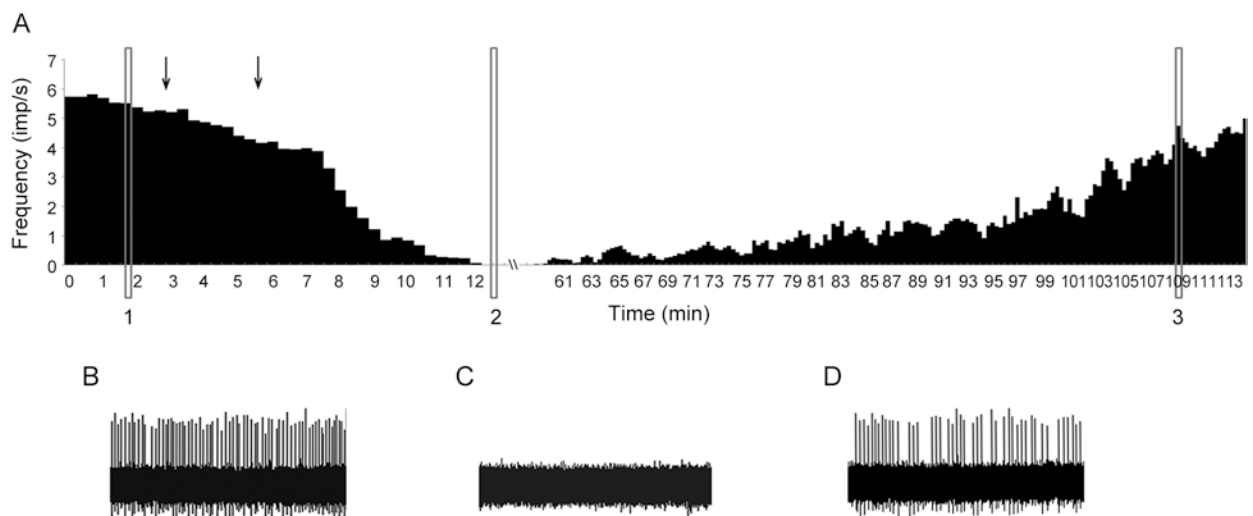
**Fig. 2.** Effect of central application of histamine on autotomy behavior in rats. Histamine (50  $\mu$ g in 5  $\mu$ L) was injected intracerebroventricularly. **A:** Progression of autotomy. **B:** Onset day of autotomy. **C and D:** The area under the curve (AUC) for days 0–21 and days 22–50 postoperatively (PO). \* $P < 0.01$ , \*\*\* $P < 0.001$  vs saline control group.



**Fig. 3.** Autotomy behavior in HDC<sup>+/+</sup> and HDC<sup>-/-</sup> mice subjected to the neuroma model. **A:** Progression of autotomy in HDC<sup>+/+</sup> and HDC<sup>-/-</sup> mice. **B:** Onset day of autotomy in the two genotypes. **C:** The area under the curve (AUC) for the period of observation. \* $P < 0.05$ .



**Fig. 4.** Effect of loratadine alone or combined with histidine on autotomy behavior in rats. Animals were intraperitoneally injected with loratadine (10 mg/kg) or a combination of histidine (500 mg/kg) and loratadine (10 mg/kg). **A:** Progression of autotomy. **B:** Onset day of autotomy. **C** and **D:** The area under the curve (AUC) for days 0–30 and days 31–50 postoperatively (PO). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  versus vehicle group.



**Fig. 5.** Blockade of spontaneous discharges in a nerve fiber from the neuroma by diphenhydramine. **A:** Histogram of firing frequency before and after drug injection. The first injection of diphenhydramine (left arrow) reduced the firing frequency. The firing gradually ceased after the second injection (right arrow) and the ectopic discharges slowly recovered in 110 min post-injection. **B–D:** Representative firing traces at 1, 2 and 3 time-points labeled in **A**.

we administered histidine systemically to increase the histamine levels in the tissues. Histidine at 500 mg/kg significantly delayed the onset of autotomy by ~11 days and inhibited the severity of autotomy, as demonstrated by the decrease in the AUC for autotomy progression by ~75%. Moreover, central application of histamine (50  $\mu$ g) also resulted in a strong inhibition of autotomy (Fig. 2). These results indicate that a sufficient increase in histamine levels suppresses spontaneous NP. The analgesic effect of histamine was additionally supported by the contrast in autotomy behavior between HDC<sup>+/+</sup> and HDC<sup>-/-</sup> C57BL/6J mice. C57BL/6J is a strain known to show low levels of autotomy. As expected, the average autotomy score in the wild-type was no higher than 1. However, HDC<sup>-/-</sup> mice, which are born with a deficiency of endogenous histamine, showed much higher autotomy scores than the wild-type (Fig. 3). These results together demonstrated that the presence of certain levels of histamine attenuates spontaneous NP.

To further explore the possible action mechanisms of histamine in spontaneous NP, we treated rats with loratadine in order to determine whether H<sub>1</sub> receptors are involved. When administered together with histidine, loratadine not only did not antagonize the analgesic effect of histidine, but even showed a tendency to inhibit autotomy more than histidine alone. Surprisingly, loratadine alone also resulted in a strong inhibition of autotomy (Fig. 4). Since loratadine is an H<sub>1</sub> receptor antagonist that poorly permeates the blood-brain barrier, which precludes central action<sup>[21]</sup>, these results suggest that, similar to its effect on acute pain<sup>[4,5]</sup> and evoked NP<sup>[7]</sup>, histamine in the periphery *via* the H<sub>1</sub> receptor pathway may contribute to the development of spontaneous NP. In addition, the electrophysiological recordings showed that another H<sub>1</sub> receptor antagonist, diphenhydramine, was able to block spontaneous discharges from the neuroma that are crucial for autotomy behavior<sup>[13,22]</sup>. It has been reported that some fibers ending in the neuroma are histamine-sensitive<sup>[23]</sup>. Therefore, H<sub>1</sub> receptor antagonists may have analgesic effects by antagonizing endogenous histamine and blocking the ectopic discharges from the neuroma.

It has been demonstrated that histamine injected into the skin elicits itching in a dose-dependent manner, and most experimental itch stimuli act *via* histamine release from mast cells<sup>[24-26]</sup>. Interestingly, histamine-induced itching

shows species-specificity. For example, histamine evokes scratching in most mouse strains but little in Sprague-Dawley rats<sup>[27,28]</sup>. It is well accepted that a specific sub-class of C-fibers mediates itch and is distinct from that mediating pain<sup>[29]</sup>. However, Baron *et al.* proposed that partial nerve injury following zoster infection might induce *de novo* expression of histamine receptors on primary nociceptive neurons or central sensitization to histaminergic stimuli, and more than that, histamine-sensitive C-fibers that are normally exclusively involved in itch transduction may gain synaptic access to pain-signaling second-order neurons in the spinal dorsal horn in the state of NP<sup>[11]</sup>. As a result, peripheral histamine-induced itch would convert into pain under neuropathic conditions. This provides further support for the present finding that histamine in the periphery is algescic after peripheral axotomy. Given that histamine in the periphery is algescic, it is very likely that the inhibitory effect of chronic histidine treatment on autotomy was largely due to its action in the CNS. It is acknowledged that histidine (500 or 1 000 mg/kg) increases the cerebral levels of histamine<sup>[30]</sup>. Therefore, we speculate that histamine in the CNS is responsible for the analgesic effect on spontaneous NP. The strong inhibition of autotomy by centrally-infused histamine clearly verified this speculation. Huang *et al.* reported that acute application of histamine elevates or decreases the threshold for evoked pain depending on the dose<sup>[8]</sup>. Some H<sub>3</sub> receptor antagonists, which increase the synthesis and release of histamine in the CNS, have been demonstrated to inhibit allodynia or hyperalgesia in animal models of evoked NP, but others do not<sup>[9,10]</sup>. Our results provide further evidence that histamine in the CNS may act as an analgesic factor in spontaneous NP, similar to that in acute pain<sup>[6,31]</sup> and evoked NP. Taken together, our results demonstrate that histamine in the periphery is algescic, whereas in the CNS it is analgesic for spontaneous NP. These data suggest that concurrent treatment with peripherally-acting H<sub>1</sub> receptor antagonists and histidine should be more effective than either alone. Actually, we did find that the combination of histidine and loratadine appeared to be the most effective regimen in suppressing autotomy. Moreover, histamine in the diet may be absorbed into the circulation and thereby increase the levels in the periphery<sup>[32]</sup>. On the contrary, H<sub>3</sub> receptor antagonists/H<sub>3</sub> inverse agonists improve histaminergic action in the CNS<sup>[33]</sup>.

Therefore, our results indicate that H<sub>3</sub> receptor antagonists/H<sub>3</sub> inverse agonists may be beneficial, while a histamine-rich diet should be avoided in patients with spontaneous NP.

We were interested to find that the inhibition of autotomy yielded by histidine, histamine or loratadine outlasted the drug-treatment period. Such a long-lasting inhibitory effect on autotomy was also reported by us and others with other drugs such as amitriptyline and ralfinamide<sup>[19,34]</sup>. Although the exact mechanisms for the long-lasting effect are not clear, these results at least suggest that the early stage after nerve injury is crucial for the development of NP. Since autotomy behavior is driven by the combination of peripheral ectopic inputs and central sensitization, these results indicate that early intervention may suppress the generation of either process, and thereby attenuate the subsequent pain abnormality. Therefore, intervention approaches should be applied opportunely after nerve injury.

In conclusion, we found that histamine in the CNS is analgesic, while that in the periphery *via* H<sub>1</sub> receptors is analgesic, in spontaneous NP. Our study justifies the avoidance of a histamine-rich diet and the use of peripherally-acting H<sub>1</sub> receptor antagonists as well as agents that improve histamine action in the CNS, such as H<sub>3</sub> receptor antagonists in patients with spontaneous NP.

## ACKNOWLEDGEMENTS

This work was supported by grants from the National Natural Science Foundation of China (81030061, 30701015, 81173042), and partly by the Foundation for Science and Technology Innovation Team, Zhejiang Province, China (2011R50014).

Received date: 2012-06-11; Accepted date: 2012-07-14

## REFERENCES

- [1] Chong MS, Bajwa ZH. Diagnosis and treatment of neuropathic pain. *J Pain Symptom Manage* 2003, 25: S4–S11.
- [2] Dieleman JP, Kerklaan J, Huygen FJ, Bouma PA, Sturkenboom MC. Incidence rates and treatment of neuropathic pain conditions in the general population. *Pain* 2008, 137: 681–688.
- [3] Hall GC, Carroll D, Parry D, McQuay HJ. Epidemiology and treatment of neuropathic pain: the UK primary care perspective. *Pain* 2006, 122: 156–162.
- [4] Farzin D, Asghari L, Nowrouzi M. Rodent antinociception following acute treatment with different histamine receptor agonists and antagonists. *Pharmacol Biochem Behav* 2002, 72: 751–760.
- [5] Kajihara Y, Murakami M, Imagawa T, Otsuguro K, Ito S, Ohta T. Histamine potentiates acid-induced responses mediating transient receptor potential V1 in mouse primary sensory neurons. *Neuroscience* 2010, 166: 292–304.
- [6] Tamaddonfard E, Rahimi S. Central effect of histamine and peripheral effect of histidine on the formalin-induced pain response in mice. *Clin Exp Pharmacol Physiol* 2004, 31: 518–522.
- [7] Zuo Y, Perkins NM, Tracey DJ, Geczy CL. Inflammation and hyperalgesia induced by nerve injury in the rat: a key role of mast cells. *Pain* 2003, 105: 467–479.
- [8] Huang L, Adachi N, Nagaro T, Liu K, Arai T. Histaminergic involvement in neuropathic pain produced by partial ligation of the sciatic nerve in rats. *Reg Anesth Pain Med* 2007, 32: 124–129.
- [9] Medhurst SJ, Collins SD, Billinton A, Bingham S, Dalziel RG, Brass A, *et al.* Novel histamine H<sub>3</sub> receptor antagonists GSK189254 and GSK334429 are efficacious in surgically-induced and virally-induced rat models of neuropathic pain. *Pain* 2008, 138: 61–69.
- [10] Smith FM, Haskelberg H, Tracey DJ, Moalem-Taylor G. Role of histamine H<sub>3</sub> and H<sub>4</sub> receptors in mechanical hyperalgesia following peripheral nerve injury. *Neuroimmunomodulation* 2007, 14: 317–325.
- [11] Baron R, Schwarz K, Kleinert A, Schattschneider J, Wasner G. Histamine-induced itch converts into pain in neuropathic hyperalgesia. *Neuroreport* 2001, 12: 3475–3478.
- [12] Suzuki R, Dickenson AH. Differential pharmacological modulation of the spontaneous stimulus-independent activity in the rat spinal cord following peripheral nerve injury. *Exp Neurol* 2006, 198: 72–80.
- [13] Wall PD, Gutnick M. Ongoing activity in peripheral nerves: the physiology and pharmacology of impulses originating from a neuroma. *Exp Neurol* 1974, 43: 580–593.
- [14] Devor M. Sensory basis of autotomy in rats. *Pain* 1991, 45: 109–110.
- [15] Zimmermann M. Pathobiology of neuropathic pain. *Eur J Pharmacol* 2001, 429: 23–37.
- [16] Ohtsu H, Tanaka S, Terui T, Hori Y, Makabe-Kobayashi Y, Pejler G, *et al.* Mice lacking histidine decarboxylase exhibit abnormal mast cells. *FEBS Lett* 2001, 502: 53–56.
- [17] Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983, 16: 109–110.
- [18] Wall PD, Devor M, Inbal R, Scadding JW, Schonfeld D, Seltzer Z, *et al.* Autotomy following peripheral nerve lesions: experimental anaesthesia dolorosa. *Pain* 1979, 7: 103–111.
- [19] Zhang SH, Blech-Hermoni Y, Faravelli L, Seltzer Z. Ralfinamide administered orally before hindpaw neurectomy or



- postoperatively provided long-lasting suppression of spontaneous neuropathic pain-related behavior in the rat. *Pain* 2008, 139: 293–305.
- [20] Raber P, Devor M. Social variables affect phenotype in the neuroma model of neuropathic pain. *Pain* 2002, 97: 139–150.
- [21] Barnett A, Iorio LC, Kreutner W, Tozzi S, Ahn HS, Gulbenkian A. Evaluation of the CNS properties of SCH 29851, a potential non-sedating antihistamine. *Agents Actions* 1984, 14: 590–597.
- [22] Devor M. Ectopic discharge in Abeta afferents as a source of neuropathic pain. *Exp Brain Res* 2009, 196: 115–128.
- [23] Seltzer Z, Paran Y, Eisen A, Ginzburg R. Neuropathic pain behavior in rats depends on the afferent input from nerve-end neuroma including histamine-sensitive C-fibers. *Neurosci Lett* 1991, 128: 203–206.
- [24] Ji RR. Recent progress in understanding the mechanisms of pain and itch. *Neurosci Bull* 2012, 28: 89–90.
- [25] Magerl W, Westerman RA, Mohnner B, Handwerker HO. Properties of transdermal histamine iontophoresis: differential effects of season, gender, and body region. *J Invest Dermatol* 1990, 94: 347–352.
- [26] Simone DA, Ngeow JY, Whitehouse J, Becerra-Cabal L, Putterman GJ, LaMotte RH. The magnitude and duration of itch produced by intracutaneous injections of histamine. *Somatosens Res* 1987, 5: 81–92.
- [27] Green AD, Young KK, Lehto SG, Smith SB, Mogil JS. Influence of genotype, dose and sex on pruritogen-induced scratching behavior in the mouse. *Pain* 2006, 124: 50–58.
- [28] Thomsen JS, Petersen MB, Benfeldt E, Jensen SB, Serup J. Scratch induction in the rat by intradermal serotonin: a model for pruritus. *Acta Derm Venereol* 2001, 81: 250–254.
- [29] Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjork HE. Specific C-receptors for itch in human skin. *J Neurosci* 1997, 17: 8003–8008.
- [30] Jochem J. Endogenous central histamine-induced reversal of critical hemorrhagic hypotension in rats: studies with L-histidine. *Shock* 2003, 20: 332–337.
- [31] Khalilzadeh E, Tamaddonfard E, Farshid AA, Erfanparast A. Microinjection of histamine into the dentate gyrus produces antinociception in the formalin test in rats. *Pharmacol Biochem Behav* 2010, 97: 325–332.
- [32] Watanabe T, Yanai K. Studies on functional roles of the histaminergic neuron system by using pharmacological agents, knockout mice and positron emission tomography. *Tohoku J Exp Med* 2001, 195: 197–217.
- [33] Arrang JM, Morisset S, Gbahou F. Constitutive activity of the histamine H3 receptor. *Trends Pharmacol Sci* 2007, 28: 350–357.
- [34] Seltzer Z, Tal M, Sharav Y. Autotomy behavior in rats following peripheral deafferentation is suppressed by daily injections of amitriptyline, diazepam and saline. *Pain* 1989, 37: 245–250.