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# Spinal histamine in attenuation of mechanical hypersensitivity in the spinal nerve ligation-induced model of experimental neuropathy

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ABSTRACT

Here we studied whether and through which mechanisms spinal administration of histamine dihydrochloride (histamine) attenuates pain behavior in neuropathic animals. Experiments were performed in rats with spinal nerve ligation-induced neuropathy and a chronic intrathecal catheter for spinal drug delivery. Mechanical hypersensitivity was assessed with monofilaments while radiant heat was used for assessing nociception. Ongoing neuropathic pain and its attenuation by histamine was assessed using conditioned place-preference test. Following spinal administration, histamine at doses 0.1–10 μg produced a dose-related mechanical antihypersensitivity effect. With prolonged treatment (twice daily 10 μg for five days), the antihypersensitivity effect of spinal histamine was reduced. In place-preference test, neuropathic animals preferred the chamber paired with histamine (10 μg). Histamine (10 μg) failed to influence heat nociception in neuropathic animals or mechanically induced pain behavior in a group of healthy control rats. Histamine-induced mechanical antihypersensitivity effect was prevented by spinal pretreatment with zolantidine (histamine $H_2$ receptor antagonist), prazosine ($\alpha_1$-adrenoceptor antagonist) and bicuculline (γ-aminobutyric acid subtype A, $GABA_A$, receptor antagonist), but not by pyrilamine (histamine $H_1$ receptor antagonist), atipamezole ($\alpha_2$-adrenoceptor antagonist), or raclopride (dopamine $D_2$ receptor antagonist). A-960656, a histamine $H_3$ receptor antagonist alone that presumably increased endogenous histamine levels reduced hypersensitivity. Additionally, histamine prevented central (presumably postsynaptically-induced) facilitation of hypersensitivity induced by N-methyl-D-aspartate. The results indicate that spinal histamine at the dose range of 0.1–10 μg selectively attenuates mechanical hypersensitivity and ongoing pain in neuropathy. The spinal histamine-induced antihypersensitivity effect involves histamine $H_2$ and $GABA_A$ receptors and (presumably neuropathy-induced) co-activation of spinal $\alpha_1$-adrenoceptors.



## 1. Introduction

The brain histaminergic system originating in the hypothalamic tuberomammillary nucleus and acting on histamine $H_1$, $H_2$, $H_3$, or $H_4$ receptors is involved in regulation of multiple functions such as sleep-waking cycle, energy and endocrine homeostasis, synaptic plasticity and learning (Haas et al., 2008; Panula et al., 2015). The brain histaminergic innervation is also involved in central regulation of pain as suggested by the following findings. Histamine has attenuated pain behavior following its administration in various brain areas including the somatosensory cortex (Tamaddonfard and Hamzeh-Gooshchi, 2014), anterior cingulate cortex (Hamzeh-Gooshchi et al., 2015), hippocampus (Erfanparast et al., 2010), and the midbrain periaqueductal gray/dorsal raphe (Thoburn et al., 1994). In general, suppression of pain behavior in these studies has been associated, although not invariably, with activation of the supraspinal histamine $H_2$ receptor. In contrast, intracerebroventricular administration of histamine $H_1$ receptor agonists has facilitated pain behavior (Farzin et al., 2002; Malmberg-Aiello et al., 1998). However, the direction of the histaminergic effect may depend on the dose, since the pain-modulatory effect has been changed from pro- to antinociception with an increase of the intracerebroventricularly administered histamine (histamine dihydrochloride) dose from $<1$ μg to $>5$ μg (Malmberg-Aiello et al., 1994).

Spinal dorsal horn that is among structures receiving histaminergic innervation from the hypothalamus (Haas et al., 2008) is a key relay for ascending pain signals and an important target for descending pain modulatory pathways. A recent series of studies has demonstrated that spinal administration of histamine at doses 800–1600 pmol (≤0.3 μg) facilitates pain behavior in healthy control animals (Sakurada et al., 2002; Watanabe et al., 2008), due

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to action on histamine $H_1$ receptors, together with other factors that include spinal glia, and tachykinin $NK_1$ and N-methyl-D-aspartate (NMDA) receptors (Mizoguchi et al., 2011; Watanabe et al., 2008). In contrast, spinal administration of histamine $H_3$ receptor agonists has reduced pain behavior in healthy controls (Cannon et al., 2003; Cannon et al., 2007b), which at least partly might be due to action on histamine $H_3$ receptors expressed by central terminals of primary afferent nerve fibers (Cannon et al., 2007a; Hough and Rice 2011).

In animals with an experimental neuropathy, locus coeruleus administration of histamine or an antagonist of the histamine $H_3$ receptor, an autoreceptor inhibiting histamine release from histaminergic nerve fibers (Arrang et al., 1983; De Luca et al., 2015), have been shown to attenuate neuropathic pain hypersensitivity (McGaraughty et al., 2012; Wei et al., 2014). However, the effect of spinal histamine or histamine receptors on neuropathic pain is not yet well known. Here we studied the contribution of spinal histamine to control of hypersensitivity and ongoing pain-like behavior in a spinal nerve ligation-induced model of peripheral neuropathy. We hypothesized that in analogy with supraspinal actions (Malmberg-Aiello et al., 1994), spinal histamine at doses $>1$ μg suppresses pain behavior. To assess the receptor mechanisms mediating the histamine-induced effect, we attempted to prevent the spinal histamine-induced effects with specific antagonists of various neurotransmitter receptors.

## 2. Materials and methods

### 2.1. Experimental animals

The experiments were performed in adult, male Hannover–Wistar rats (weight: 180–230 g; Harlan, Horst, The Netherlands). The experimental protocol was accepted by the Ethical Committee on Animal Experiments of the regional government of Southern Finland. The experiments were performed according to the guidelines of European Communities Council Directive 2010/63/EU on the use of animals for scientific purposes. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

### 2.2. Drugs

Histamine dihydrochloride, pyrilamine maleate (histamine $H_1$ receptor antagonist), zolantidine (histamine $H_2$ receptor antagonist), bicuculline (γ-aminobutyric acid subtype A, $GABA_A$, receptor antagonist), N-methyl-D-aspartate (NMDA), raclopride (dopamine D2 receptor antagonist), WAY-100635 ($5\text{-}HT_{1A}$ receptor antagonist) and prazosine ($\alpha_1$-adrenoceptor antagonist) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Histamine dihydrochloride was dissolved in physiological saline, titrated to a pH between 6.0 and 6.5 with NaOH and diluted with saline (Thoburn et al., 1994). For the sake of brevity, the word histamine is used when referring to spinal administration of histamine dihydrochloride elsewhere in the text. A-960656, a histamine $H_3$ receptor antagonist, was generously provided by Abbvie (North Chicago, IL, U.S.A.) (Cowart et al., 2012; Hsieh et al., 2010b). A-960656 was dissolved in a vehicle of 10% dimethylsulfoxide (DMSO)/90% hydroxy-β-cyclodextrin. Atipamezole ($\alpha_2$-adrenoceptor antagonist) was purchased from OrionPharma (Turku, Finland). Atipamezole is selective for $\alpha_2$-adrenoceptors but not their subtypes (Pertovaara et al., 2005). Physiological saline was used as control, except that 10% DMSO/90% hydroxy-β-cyclodextrin was used as a control for A-960656.

The choice of doses was based on earlier studies on histamine [1–3 μg (Wei et al., 2014)], zolantidine [10 μg (Wei et al., 2014)], bicuculline [0.03 μg (Wei et al., 2011)], NMDA [200 ng (Wei et al., 2011)], prazosine [30 μg (Wei et al., 2014)], atipamezole [5 μg [Wei and Pertovaara, 2006)], raclopride [1 μg (Viisanen et al., 2012)], and WAY-100635 [3 μg (Pertovaara and Wei, 2008)]. Preliminary studies together with literature search were performed to choose the doses of receptor antagonists, such as A-960656 [⩾30 μg (Cowart et al., 2012; Hsieh et al., 2010b)] or pyrilamine [10 μg (Chung et al., 1984)].

### 2.3. Techniques for producing neuropathy

There are a number of surgically induced models of peripheral neuropathy (Honoré et al., 2011), of which we chose for this study the spinal nerve ligation (SNL) model. The unilateral ligation of two spinal nerves (L5 and L6) was performed under pentobarbitone anesthesia (60 mg/kg intraperitoneally) as described in detail earlier (Kim and Chung, 1992; Röyttä et al., 1999). Briefly, the left L5 and L6 spinal nerves were isolated and tightly ligated with 6–0 silk thread. Only nerve-injured animals with tactile allodynia-like hypersensitivity (hind limb withdrawal threshold to monofilament stimulation in the operated side $<6$ g, which is below the lower 95% confidence limit of the threshold in unoperated control animals) were selected for this study. Nerve-injured animals were tested two to three weeks after the operation. For comparison, in one experiment a group of healthy control animals was studied.

### 2.4. Techniques for intrathecal drug injections

For intrathecal (i.t.) drug injections, a catheter (PE-10; Becton Dickinson and Company, Sparks, MD, U.S.A.) was administered into the lumbar level of the spinal cord under pentobarbitone anesthesia (60 mg/kg intraperitoneally) as described in detail elsewhere (Størkson et al., 1996). Following recovery from anesthesia, the correct placing of the catheter was verified by administering lidocaine (4%, 7–10 μl followed by a 15 μl of saline for flushing) with a 50 μl Hamilton syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland). Only those rats that had no motor impairment before lidocaine injection but had a bilateral paralysis of hind limbs following i.t. administration of lidocaine were studied further. The lidocaine test was performed at least 3 days prior to the start of the drug testing sessions. For i.t. administration the drugs were microinjected with a 50 μl Hamilton microsyringe at a volume of 5–7 μl followed by a saline flush at a volume of 15 μl.

### 2.5. Behavioral testing of mechanical hypersensitivity and heat nociception

In the currently used model of peripheral neuropathy, mechanical hypersensitivity is common and often robust. Therefore, the focus of this study was in the assessment of tactile allodynia-like hypersensitivity by determining a limb withdrawal response evoked by monofilament stimulation of the injured dermatome. To find out whether histamine produced a more wide-spread influence on nociception, heat nociception was assessed in one experiment.

Prior to any testing, the rats were habituated to the experimental conditions by allowing them to spend 1–2 h daily in the laboratory during 2–3 days. For assessment of tactile allodynia-like hypersensitivity, the hind limb withdrawal threshold evoked by stimulation of the hind paw with monofilaments (von Frey-hairs) was determined while the rat was standing on a metal grid. At each time point, the paw ipsilateral to the spinal nerve ligation was stimulated five times with an ascending series of calibrated monofilaments (in neuropathic animals 1–26 g, and in healthy controls 1–60 g; North Coast Medical, Inc., Morgan Hill, CA, U.S.A.). At each stimulus force, the withdrawal response frequency was

determined. An increase in the withdrawal response rate was considered to represent mechanical hypersensitivity effect. When compared with the traditional determination of the withdrawal threshold value, the currently used method has the advantage that it allows assessing separately drug effects on withdrawal responses evoked by stimulus forces of threshold and suprathreshold levels. While the testing was not formally blinded, our previous study by the same experimenter showed that the drug-induced mechanical antihypersensitivity effect using the same test stimulus procedure in the rat was of identical magnitude with and without formal blinding (Wei et al., 2012).

For assessment of thermal nociception in the plantar skin of the hind paw, the latency of the heat-induced limb withdrawal response was determined using a radiant heat device (Plantar test model 7370, Ugo Basile, Varese, Italy). To avoid tissue damage, two consecutive measurements at each time point were made at one min intervals. The mean latency at each time point was used in further calculations. The stimulus intensity was adjusted so that the mean baseline latency was 8–9 s and the cut-off latency was 15 s. Neuropathy-induced changes in skin temperature may provide a confounding factor in the assessment of radiant heat-induced withdrawal latencies (Luukko et al., 1994). To exclude skin temperature-related changes as a cause of withdrawal latency changes, hind paw skin temperature was assessed with an electronic thermometer (BAT-12, Physitemp Instruments Inc., Clifton, NJ, U.S.A.) before and after drug administration just prior to delivery of each heat stimulus.

### 2.6. Conditioned place-preference test

Conditioned place-preference test (King et al., 2009; Sufka, 1994) was used for assessing ongoing neuropathic pain and its attenuation by spinal administration of histamine using procedure described in detail elsewhere (Wei et al., 2013). Briefly, rats underwent a 3 day habituation, in which they were placed in automated CPP boxes (Place Preference System, San Diego Instruments, Inc., San Diego, CA, U.S.A.) with access to all 3 chambers for 30 min per day during the first two days. The device records time spent in each chamber using a computer-controlled $4 \times 16$ array of photo beams. Among differences between the test chambers was the roughness of the floor (rough versus smooth) and the painting of the walls (black triangles versus bars on white surface). Time spent in each of the boxes was recorded for 15 min on day 3 (D3). Rats that spent more than 720 s in one of the conditioning chambers were eliminated from the study. The following day (D4), all rats received a morning injection of saline and were placed in one of the pairing chambers for 30 min. Four hours later, all rats received histamine (10 μg) and were placed in the opposite chamber for 30 min. On the next day (D5), 20 h following drug pairing, animals were placed drug-free in the place-preference boxes with access to all chambers. The amount of time spent in each of the two chambers (saline- and histamine-paired) was automatically registered and used to quantify the conditioning effect by drug treatment. It was expected that if the animal had ongoing pain that was reduced by histamine treatment, the animal preferred the histamine-paired chamber.

### 2.7. Motor performance

To exclude a motor effect of histamine, motor activity of the rats was assessed in the Rotarod test using a commercially available device (Ugo Basile). The revolution speed was 12 revolutions per minute (rpm). The rats were put on the drum 15 min after i.t. administration of 10 μg of histamine or vehicle on separate days in a counterbalanced order. The time animals were able to stay on the drum was calculated. Cut off time was 60 s.

### 2.8. Course of the study

Animals were tested two to three weeks after spinal nerve ligation and administration of the intrathecal catheter. In each acute drug testing condition, the assessment of mechanical hypersensitivity was performed before and at various time points up to 30 min following drug administrations. Each drug condition was tested on a separate day. Mechanical and heat sensitivity were assessed on separate days. In attempts to prevent the histamine-induced effect, the spinal cord was pretreated with the studied receptor antagonist before histamine administration; the delay between administration of the compound used for the prevention attempt and histamine administration was 3 min. The delay was chosen based on previous behavioral investigations using the studied compounds so that both compounds were expected to have their maximal effects at about the same time. The effects of drugs used in prevention attempts were tested also alone at the same dose as in prevention attempts. The maximum effects of treatments were chosen for further analyses. Each animal participated in two to six acute drug testing sessions at an interval of 2–4 days and counterbalanced order. Assessment of pre-drug responses in each acute drug testing session indicated that none of the drug treatments had long-term effects.

Conditioned place-preference test was performed in drug-naive animals as described in Section 2.6. Effects of histamine versus vehicle on motor performance were assessed in a counterbalanced order on separate days using Rotarod test as described in Section 2.7.

When assessing the antihypersensitivity effect of prolonged treatment with histamine, one drug-naive group of neuropathic animals was treated i.t. with 10 μg of histamine twice daily for five days, while the comparison group was treated twice daily with vehicle. In both groups, mechanical hypersensitivity was assessed before and 15 min after the first and last administration of drug/vehicle.

At the end of the experiments, the animals were sacrificed by giving a lethal dose of pentobarbitone.

### 2.9. Statistical analyses

Statistical evaluation of the data was performed using one- or two-way analysis of variance followed by Tukey's test, or with *t*-test when comparing two groups. $P < 0.05$ (two-tailed) was considered to represent a significant difference.

## 3. Results

### 3.1. Attenuation of pain behavior by spinally administered histamine

When administered i.t., histamine produced mechanical antihypersensitivity effect that was dose-related (0.1–10 μg tested at the stimulus force of 8 g; main effect of dose: $F_{4,33}=4.51$, $P=0.005$; Fig. 1A). *Post hoc* testing indicated that the lowest dose of histamine producing a significant mechanical antihypersensitivity effect was 10 μg (Fig. 1A). The maximum mechanical antihypersensitivity effect was reached within 15 min, and the duration of the antihypersensitivity effect was $>30$ min (Fig. 1B). To assess whether the histamine-induced antihypersensitivity effect varies with the test stimulus intensity, the effect of histamine was determined at various test stimulus forces. The mechanical antihypersensitivity effect induced by histamine (10 μg) varied with the intensity of mechanical test stimulation (interaction between the test stimulus force and drug treatment: $F_{7,80}=4.77$, $P=0.0002$; Fig. 1C). *Post hoc* testing indicated that at the lowest test stimulus forces of 1 g and 2 g that activate selectively only

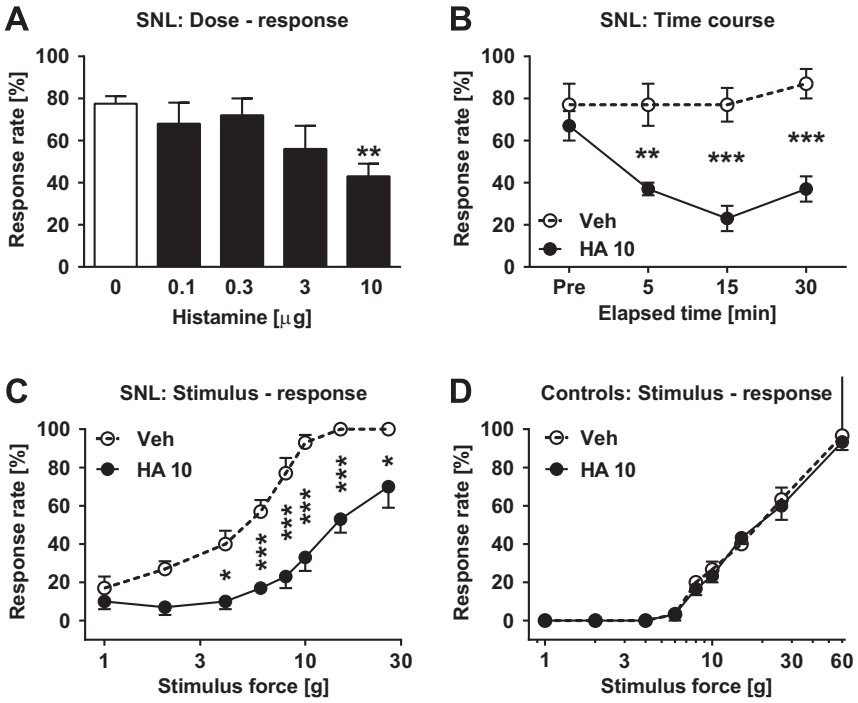


**Fig. 1.** General characteristics of the mechanical antihypersensitivity effect induced by histamine (HA) in the spinal cord. (A) The dose-dependence of the mechanical antihypersensitivity effect of histamine. (B) Time course of the mechanical antihypersensitivity effect induced by histamine. (C) The antihypersensitivity effect of histamine at various test stimulus intensities. (D) Attenuation of mechanical nociception by histamine. HA 0.1–10 represent intrathecally administered histamine doses in μg. Veh, vehicle. SNL, spinal nerve ligation. Test stimulus force in A and B was 8 g. Except for B, testing was performed 15 min after spinal histamine/vehicle administration. In A–C, animals were nerve-injured and in D, healthy controls. Decreases in the response rate represent antihypersensitivity effect in A–C and antinociceptive effect in D. Error bars represent S.E.M. ($n=6$, except in A, $n_0=16$, and $n_{0.1\ \&\ 0.3}=5$). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.005$ (Tukey's test; reference: the corresponding Veh-value).

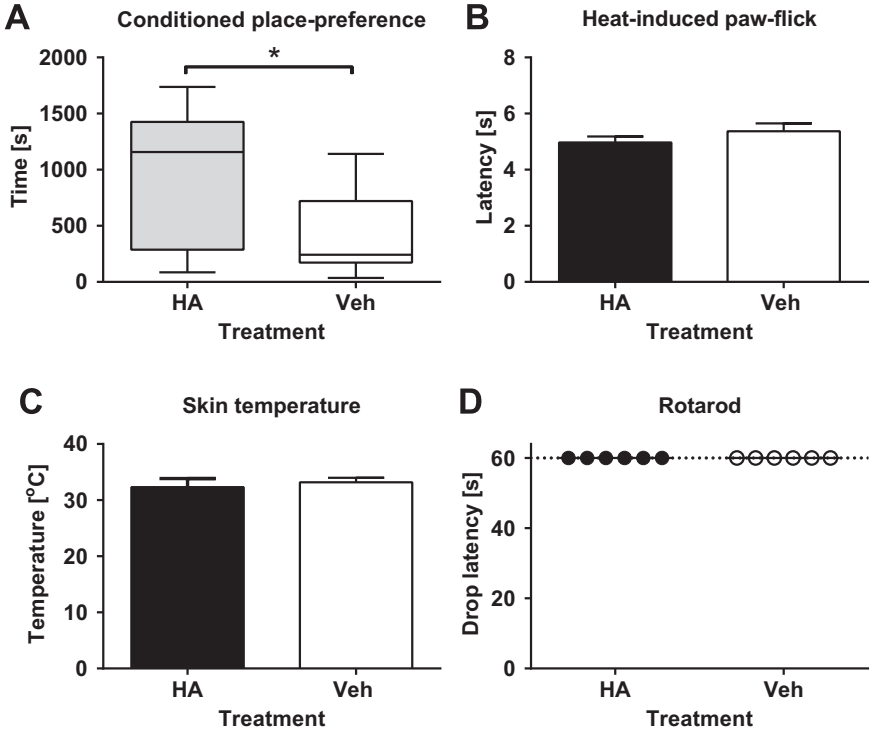


**Fig. 2.** Effect of spinally administered histamine on spontaneous neuropathic pain-like behavior (A), heat nociception (B), paw skin temperature (C), and motor performance (D) in animals with a spinal nerve ligation-induced neuropathy.Histamine (HA) dose in each graph was 10 μg, and testing was performed 5–20 min (A) or 15 min (B–D) after the treatment with HA or vehicle (Veh). In A, an increase of time spent in the HA-paired chamber is considered to represent reduction of ongoing neuropathic pain-like behavior. In A, the boxes represent the median and its interquartile ranges, whereas the whiskers represent the range ($n=15$). In B and C, the mean and S.E.M. are shown ($n=6$). In D, the symbols represent individual animals and the dotted horizontal line represents the cut-off latency ($n=6$). $^{*}P<0.05$ (paired $t$-test).

mechanoreceptors the histamine-induced suppressive effect was not significant. At test stimulus forces ⩾4 g (up to the force of 26 g that was the maximum used in nerve-injured animals of the present study) that recruited at least partly mechanonociceptors as well as mechanoreceptors (Leem et al., 1993) the histamine-induced antihypersensitivity effect was significant (Fig. 1C).

To find out whether the effect of histamine on mechanically evoked pain behavior depends on the pathophysiological condition, the effect of i.t. histamine was assessed in healthy controls at the dose of 10 μg i.t. that produced a significant antihypersensitivity effect in nerve-injured animals failed to attenuate responses to mechanical stimulation in healthy controls (main effect of drug: $F_{1,90}=0.52$), independent of the test stimulus intensity (interaction between test stimulus intensity and drug treatment: $F_{8,90}=0.26$; Fig. 1D).

To assess whether spinal histamine attenuates ongoing pain behavior in neuropathy, nerve-injured rats were tested using conditioned place-preference paradigm. Neuropathic animals spent significantly more time in the test chamber paired earlier with spinal administration of 10 μg histamine than in the chamber paired with spinal administration of vehicle ($t_{14}=2.16$, $P=0.049$; Fig. 2 A).

Radiant heat-induced paw-flick latency was used to assess thermal nociception. Before drug treatments, the heat-evoked response latency was significantly shorter in the nerve-injured limb (5.4+0.3 s;+S.E.M., $n=6$) than in the contralateral limb (6.1+0.3 s; $t_5=4.74$, $P=0.005$), which, however, may, at least be partly explained by the significantly higher skin temperature of the injured than the contralateral limb (33.2+0.8 °C versus 31.4+0.8 °C; $t_5=6.2$, $P=0.002$). Effect of histamine on thermal nociception was assessed in the injured limb. When compared with vehicle treatment, spinal administration of 10 μg of histamine failed to influence thermal nociception ($t_5=1.97$; Fig. 2B) or skin temperature ($t_5=0.59$; Fig. 2C) in the nerve-injured limb. Spinal administration of 10 μg of histamine had no marked effect on motor behavior of neuropathic animals in the Rotarod test. All animals were able to stay on the revolving Rotarod drum until the maximum of 60 s, independent whether they were treated with histamine or vehicle (Fig. 2D).

### 3.2. Spinal histamine receptors mediating the antihypersensitivity effect

I.t. pretreatment of the lumbar spinal cord with the histamine $H_1$ receptor antagonist pyrilamine (10 μg) failed to attenuate the mechanical antihypersensitivity effect induced by i.t. administration of histamine (10 μg; main effect of drugs: $F_{3,18}=23.7$, $P<0.0001$; Fig. 3 A), whereas i.t. pretreatment with the histamine $H_2$ receptor antagonist zolantidine (10 μg) significantly attenuated the antihypersensitivity effect induced by spinal histamine (10 μg; main effect of drugs: $F_{3,20}=22.9$, $P<0.0001$; Fig. 3B). Pyrilamine or zolantidine alone at the currently used i.t. doses (10 μg and 10 μg, respectively) failed to influence mechanical hypersensitivity (Fig. 3 A and B).

I.t. administration of the selective histamine $H_3$ receptor antagonist A-960656 alone produced a dose-related (0 μg, 30 μg, 100 μg) antihypersensitivity effect (main effect of dose:

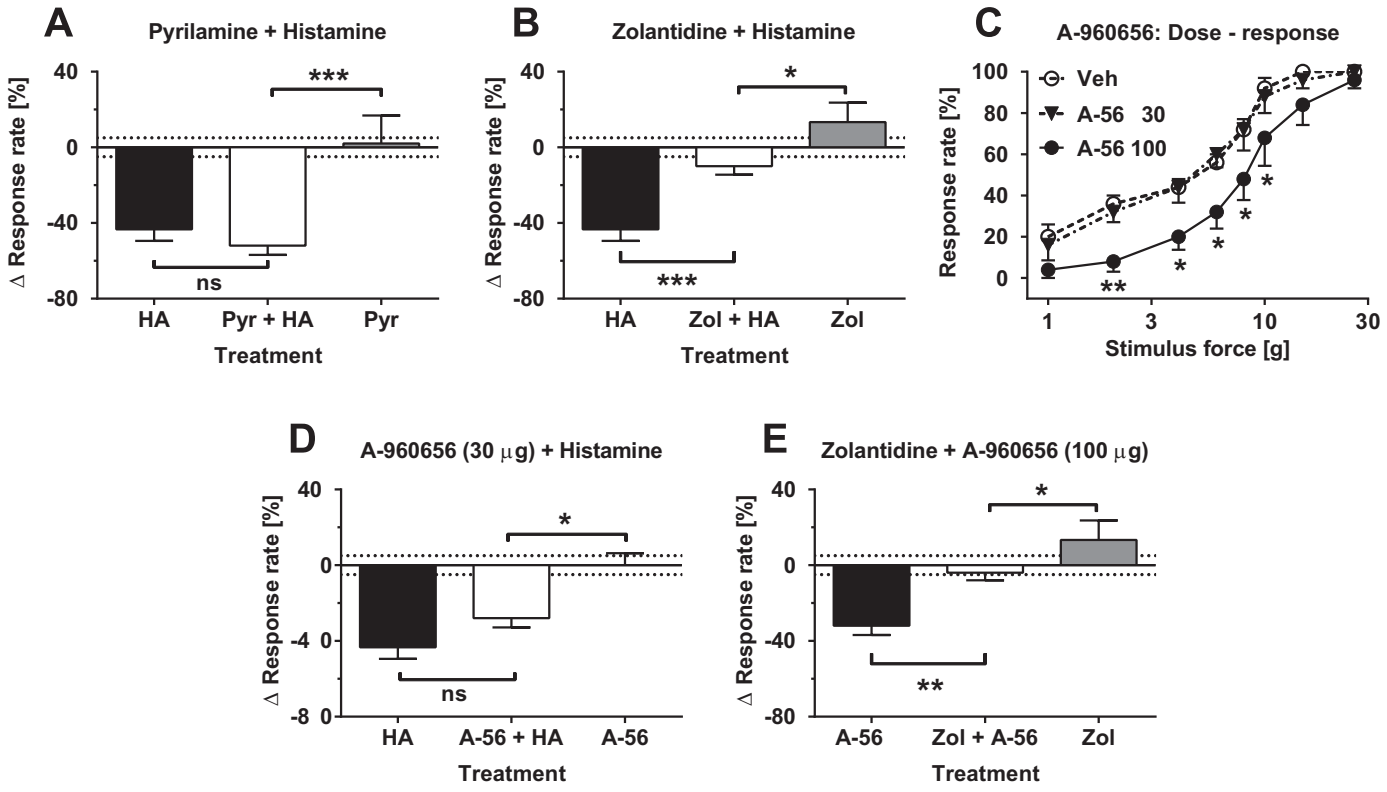


**Fig. 3.** Roles of various histamine receptors in mediation of the spinal histamine-induced mechanical antihypersensitivity effect. (A) Mechanical antihypersensitivity effect induced by histamine (HA; 10 μg) and its attempted prevention by spinal pretreatment with pyrilamine (Pyr; 10 μg), a histamine $H_1$ receptor antagonist. (B) Prevention of the histamine-induced mechanical antihypersensitivity effect by spinal pretreatment with zolantidine (Zol; 10 μg), a histamine $H_2$ receptor antagonist. (C) A dose-related antihypersensitivity effect induced by spinal treatment with A-960656 (A-56), a histamine $H_3$ receptor antagonist, alone. (D) Mechanical antihypersensitivity effect induced by histamine (10 μg) and its attempted prevention by spinal pretreatment with A-960656 at a low dose (30 μg) that alone was ineffective. (E) Prevention of the antihypersensitivity effect induced by a high (100 μg) dose of A-960656 by spinal pretreatment with zolantidine (10 μg). Veh=vehicle. Mechanical hypersensitivity was assessed 15 min after drug administrations at a stimulus force of 8 g. *Y*-axis shows the difference in response rate when compared with the corresponding pre-drug rate (pre-treatment response rate – post-treatment response rate). A response rate difference < 0 represents antihypersensitivity effect. Error bars represent S.E.M. (in A, C, D, and E $n=5$–6, in B, $n=6$). The dotted horizontal lines in A, B, D, and E represent 95% confidence intervals (CIs) of the response rate change in vehicle-treated animals ($n=6$). The 95% CIs extended from 5% to −5% in A, B, D, and E. ns=not significant, $^{*}P<0.05$, $^{**}P<.01$, $^{***}P<0.005$ (Tukey's test; reference in C: the corresponding Veh-value).

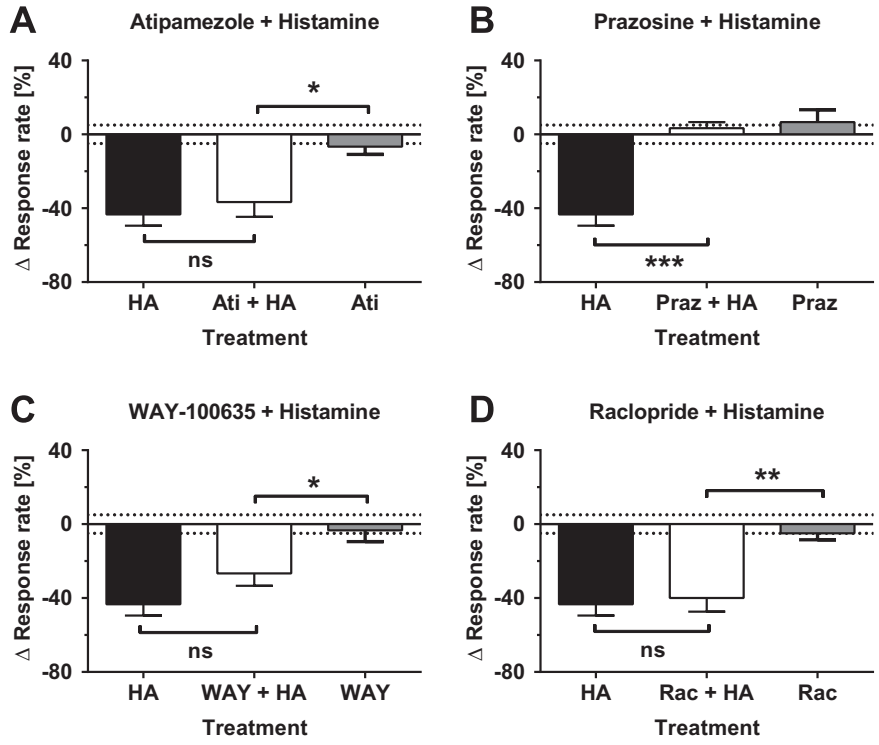


**Fig. 4.** Roles of various monoaminergic receptors in mediation of the spinal histamine-induced mechanical antihypersensitivity effect. (A) Mechanical antihypersensitivity effect induced by histamine (HA; 10 μg) and its attempted prevention by spinal pretreatment with atipamezole (Ati; 5 μg), an $\alpha_2$-adrenoceptor antagonist. (B) Prevention of the histamine-induced mechanical antihypersensitivity effect by spinal pretreatment with prazosine (Praz; 30 μg), an $\alpha_1$-adrenoceptor antagonist. (C) Mechanical antihypersensitivity effect induced by histamine and its attempted prevention by spinal pretreatment with WAY-100635 (WAY; 3 μg), a 5-$HT_{1A}$ receptor antagonist. (D) Mechanical antihypersensitivity effect induced by histamine and its attempted prevention by spinal pretreatment with raclopride (Rac; 1 μg), a dopamine D2 receptor antagonist. Main effect of drug was significant in all conditions (A: $F_{3,20}=12.6$, $P<0.0001$; B: $F_{3,20}=18.4$, $P<0.0001$; C: $F_{3,20}=11.4$, $P=0.0001$; D: $F_{3,20}=16.0$, $P<0.0001$). Veh = vehicle. Mechanical hypersensitivity was assessed at a stimulus force of 8 g. *Y*-axis shows the difference in response rate when compared with the corresponding pre-drug rate (pretreatment response rate – post-treatment response rate). A response rate difference $<0$ represents antihypersensitivity effect. Error bars represent S.E.M. (in all graphs, $n=6$). The dotted horizontal lines represent 95% confidence intervals (CIs) of the response rate change in vehicle-treated animals. ns = not significant, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.005$ (Tukey's test).

$F_{2,96}=22.95$, $P<0.0001$; Fig. 3C). The maximum antihypersensitivity effect induced by 100 μg of A-960656 was obtained within 5 min and the antihypersensitivity effect stayed at the same level at least up to 30 min (not shown). *Post hoc* testing indicated that pretreatment of the spinal cord with A-960656 at a dose of 30 μg that alone had no significant effect on mechanical hypersensitivity failed to produce a significant reduction of the mechanical antihypersensitivity effect induced by 10 μg of histamine (main effect of drugs in the experimental condition, however, was significant: $F_{3,18}=14.8$, $P<0.0001$; Fig. 3D). The antihypersensitivity effect induced by a high dose (100 μg) of A-960656 was prevented by pretreatment with 10 μg of zolantidine (main effect of drugs: $F_{3,18}=16.4$, $P<0.0001$; Fig. 3E).

### 3.3. Spinal monoaminergic receptors in the histamine-induced antihypersensitivity effect

Blocking the spinal $\alpha_2$-adrenoceptors with atipamezole (5 μg) failed to prevent the mechanical antihypersensitivity effect induced by 10 μg of spinally administered histamine (Fig. 4A). In contrast, blocking the spinal $\alpha_1$-adrenoceptors with prazosine (30 μg) completely prevented the mechanical antihypersensitivity effect induced by 10 μg of spinally administered histamine (Fig. 4B). Blocking the spinal 5-$HT_{1A}$ receptors with WAY-100635 (3 μg; Fig. 4C) or spinal dopamine D2 receptors with raclopride (1 μg; Fig. 4D) failed to prevent the mechanical antihypersensitivity effect induced by 10 μg of spinally administered histamine. At the currently used doses, atipamezole, prazosine, WAY-100635 or raclopride failed to have a significant influence on mechanical hypersensitivity (Fig. 4 A–D).

### 3.4. Spinal $GABA_A$ receptors in the histamine-induced hypersensitivity effect

To assess whether spinal $GABA_A$ receptors are involved in the histamine-induced antihypersensitivity effect, bicuculline (a $GABA_A$ receptor antagonist) was administered i.t. at the dose of 0.03 μg that alone had no effect on hypersensitivity (Fig. 5A). Mechanical antihypersensitivity effect induced by spinal administration of 10 μ of histamine was completely prevented by bicuculline (Fig. 5A).

### 3.5. Effect of histamine on centrally induced facilitation of hypersensitivity

To assess whether spinal histamine attenuates hypersensitivity induced by central (presumably postsynaptic) facilitation of spinal pain-relay neurons, 200 ng of NMDA was administered spinally. NMDA co-administered with vehicle significantly facilitated mechanical hypersensitivity in nerve-injured animals as indicated by the finding that the lower 95% confidence interval of the response elicited in the group treated with a combination of NMDA and vehicle was above the upper 95% confidence interval of the response in the group treated with vehicle alone (Fig. 5 B). Co-administration of histamine at the dose of 10 μg prevented the NMDA-induced central facilitation of hypersensitivity (Fig. 5 B).

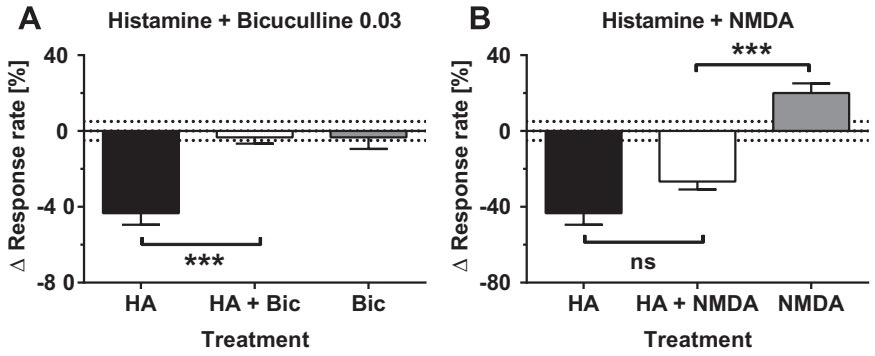


**Fig. 5.** Interactions of the spinal $GABA_A$ and NMDA receptors with the spinal histamine-induced mechanical antihypersensitivity effect in animals with peripheral neuropathy. (A) Spinally administered bicuculline (Bic; 0.03 μg), a $GABA_A$ receptor antagonist, prevents the mechanical antihypersensitivity effect induced by histamine (HA; 10 μg). (B) Attenuation of the spinal NMDA (0.1 μg) induced central facilitation of mechanical hypersensitivity by spinal administration of histamine.
Main effect of drug was significant in both conditions (A: $F_{3,20}=15.0$, $P<0.0001$; B: $F_{3,20}=28.9$, $P<0.0001$). Veh=vehicle. Mechanical hypersensitivity was assessed at a stimulus force of 8 g. *Y*-axis shows the difference in response rate when compared with the corresponding pre-drug rate (pretreatment response rate – post-treatment response rate). A response rate difference $<0$ represents antihypersensitivity effect. Error bars represent S.E.M. (in all graphs, $n=6$). The dotted horizontal lines represent 95% confidence intervals (CIs) of the response rate change in vehicle-treated animals. ns=not significant, $^{***}P<0.005$ (Tukey's test).

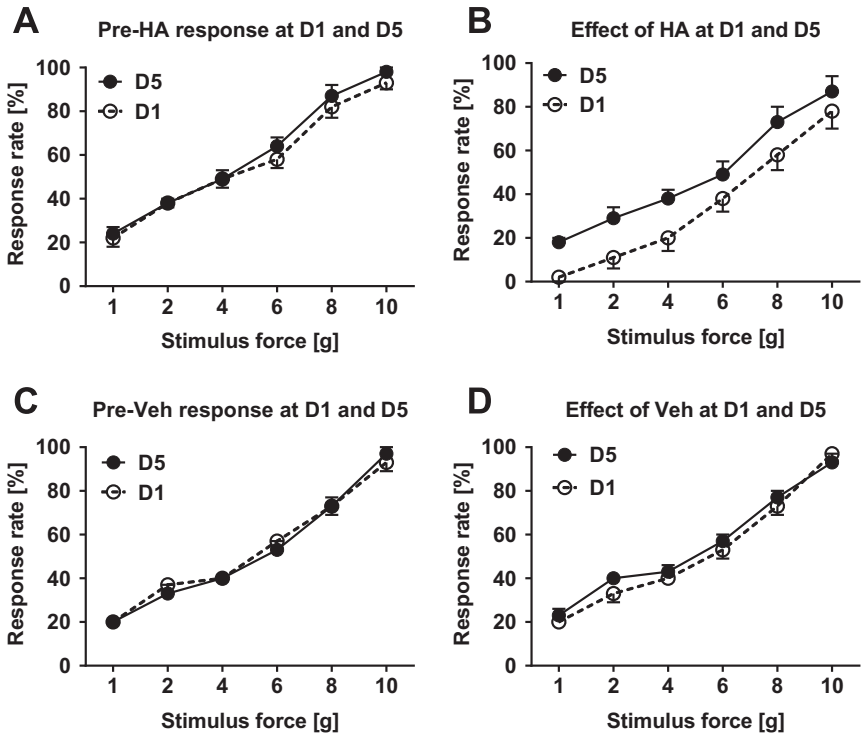


**Fig. 6.** Influence by prolongation of the treatment period from one to five days on spinal histamine-induced antihypersensitivity effect. Histamine (10 μg) or vehicle (Veh) was administered intrathecally twice daily for five days. The mechanical antihypersensitivity effect was assessed on the first (D1) and last (D5) treatment day before (A) and 15 min after administration of histamine (B), and before (C) and 15 min after administration of vehicle (D). Decreases in the response rate represent antihypersensitivity effect. Error bars represent S.E.M. ($n=6$).

### *3.6. Antihypersensitivity effect following prolonged treatment with histamine*

To assess whether the magnitude of the antihypersensitivity effect induced by spinally administered histamine is changed with prolonged treatment, 10 μg of histamine was administered twice daily for five days. Baseline hypersensitivity and the mechanical antihypersensitivity effect of histamine was assessed on day 1 (D1) and D5. Baseline response rates to mechanical stimulation (i.e., before histamine treatment) were identical on D1 and D5 (main effect of treatment day: $F_{1,96}=2.03$; Fig. 6A). However, the mechanical antihypersensitivity effect induced by histamine was significantly reduced on D5 (main effect of treatment day: $F_{1,96}=19.26$, $P<0.0001$; Fig. 6B). Prolonged vehicle treatment had no influence on baseline hypersensitivity (main effect of treatment day: $F_{1,60}=0.14$; Fig. 6C) or hypersensitivity after vehicle administration (main effect of treatment day: $F_{1,60}=2.65$; Fig. 6D).

## 4. Discussion

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In the present study, spinal histamine at a dose of 10 μg reduced pain hypersensitivity and ongoing pain in neuropathy, while it had no effect on mechanically induced pain behavior in healthy controls. The suppression of pain behavior in neuropathic animals was submodality-selective, since the dose of histamine producing an attenuation of mechanical hypersensitivity and

ongoing pain failed to influence heat nociception. While histamine is able to depress spinal motoneurons (Phillis et al., 1968), the failure to influence locomotor behavior or heat nociception at a dose that attenuated mechanical hypersensitivity indicates that the antihypersensitivity effect was due to a selective depression of spinal sensory rather than motoneurons.

The finding that blocking the spinal $H_2$ but not $H_1$ or $H_3$ receptor prevented the spinal histamine-induced antihypersensitivity effect suggests that the histamine $H_2$ receptor was mediating the antihypersensitivity effect in the spinal cord. Earlier findings indicate that spinally administered histamine $H_3$ agonist attenuates mechanical nociception in healthy control animals (Cannon et al., 2003), presumably due to presynaptic action on spinal terminals of primary afferent nerve fibers (Hough and Rice, 2011). In neuropathic animals of the present study, in contrast, intrathecal injection of the $H_3$ receptor antagonist alone attenuated hypersensitivity that could be prevented by spinal delivery of an $H_2$ receptor antagonist in the current study. This finding might be explained by increased release of endogenous histamine due to reduced autoinhibition of histaminergic nerve terminals. Since a high dose of the histamine $H_3$ receptor antagonist was needed to produce a significant antihypersensitivity effect following spinal administration while a low dose has been effective in the pontine locus coeruleus (Wei et al., 2014), it is possible that diffusion to the brain contributed to the antihypersensitivity effect induced by the spinally administered histamine $H_3$ receptor antagonist alone.

At doses considerably lower than those having an antihypersensitivity effect, spinally administered histamine has produced a histamine $H_1$ receptor-mediated facilitation of pain behavior in healthy controls (Mizoguchi et al., 2011; Sakurada et al., 2002; Watanabe et al., 2008; Yoshida et al., 2005). The low-dose histamine-induced pronociceptive mechanism is likely to be saturated in neuropathic animals, since histamine at doses 0.1–0.3 μg did not induce a further facilitation of hypersensitivity.

Spinal pretreatment with an antagonist of the $\alpha_1$-adrenoceptor or the $GABA_A$ receptor at doses that themselves had no effects prevented the spinal histamine-induced antihypersensitivity effect. However, the spinal pretreatment with an antagonist of the dopamine D2 receptor or the $\alpha_2$-adrenoceptor failed to attenuate mechanical antihypersensitivity effect induced by spinally histamine. Moreover, pretreatment with an antagonist of the 5-$HT_{1A}$ receptor failed to produce a significant attenuation of the histamine-induced antihypersensitivity effect, although due to apparent tendency to a reduction of the histamine-induced antihypersensitivity effect one should be cautious with this finding. Together these results suggest that interactions with descending serotonergic or dopaminergic pathways do not have a critical contribution to the spinal histamine-induced antihypersensitivity effect, whereas the spinal $GABA_A$ receptor and the spinal $\alpha_1$- but not $\alpha_2$-adrenoceptor are involved in the histamine-induced antihypersensitivity effect.

GABAergic neurons have an important role in the attenuation of pain-related signals in the spinal dorsal horn (Braz et al., 2014). GABAergic neurons, at least in the septohippocampal area, are known to be activated by a direct histamine $H_2$ receptor-mediated action (Xu et al., 2004). These earlier findings are in line with the proposal that histamine $H_2$ receptor-mediated direct activation of inhibitory GABAergic neurons contributed to the spinal histamine-induced antihypersensitivity effect. Additionally or alternatively, histamine may also directly activate $GABA_A$ receptors (Saras et al., 2008). The direct action of histamine on $GABA_A$ receptors, however, has not been specifically blocked by antagonists of the histamine $H_2$ receptor (Saras et al., 2008), unlike the histamine-induced antihypersensitivity effect in the present study. Therefore, a histamine $H_2$ receptor-mediated activation of GABAergic neurons may have a more important role in the antihypersensitivity effect than a direct histaminergic action on postsynaptic $GABA_A$ receptors, although it is possible that both of these mechanisms contributed to the antihypersensitivity effect induced by histamine.

While spinal GABAergic neurons may attenuate pain-related signals by a direct postsynaptic action on pain-relay neurons, they may also presynaptically suppress central terminals of primary afferent nerve fibers (Yuan et al., 2009). Here, a direct postsynaptic effect of GABAergic neurons driven by histamine was likely to be the predominant mechanism of the antihypersensitivity effect, since histamine prevented the presumably postsynaptic increase of hypersensitivity induced by spinally administered NMDA equally well as it prevented hypersensitive responses evoked by peripheral (presynaptic) stimulation.

The finding that only the $\alpha_1$- but not the $\alpha_2$-adrenoceptor antagonist prevented the histamine-induced antihypersensitivity effect was unexpected. Namely, if spinally administered histamine increased release of noradrenaline from all spinal nerve terminals of descending noradrenergic pathways resulting in pain attenuating effect the co-existence of which was necessary for the histamine-induced antihypersensitivity effect, it might have been expected that also blocking the spinal $\alpha_2$-adrenoceptors had prevented the histamine-induced effect, since both the spinal $\alpha_1$- and $\alpha_2$-adrenoceptor are involved in noradrenergic suppression of pain-related signals (see for references Pertovaara, 2013). A possible explanation for the selective contribution of the $\alpha_1$-adrenoceptor is that the spinal GABAergic neuron expresses only the $\alpha_1$-adrenoceptor through which descending noradrenergic pathways innervating the GABAergic neuron can drive it (Baba et al., 2000 a, b; Gassner et al., 2009; Millar and Williams, 1989). While the descending noradrenergic system is only weakly, if at all activated in healthy controls (Mansikka et al., 2004), earlier behavioral studies suggest that even without spinal administration of histamine peripheral neuropathy may induce a tonic drive of descending noradrenergic pathways (Hughes et al., 2013; Wei and Pertovaara, 2006; Xu et al., 1999). In line with this, it has been shown that neuropathy induces an increased firing response to peripheral stimulation (Alba-Delgado et al., 2012; Viisanen and Pertovaara, 2007) and an increased metabolic activity in the locus coeruleus (Brightwell and Taylor, 2009; Mao et al., 1993). Moreover, an increased spinal noradrenaline level has been described in neuropathy (Hayashida et al., 2008; however, Song, et al., 2013). It may be speculated that without an accompanying neuropathy-induced facilitation of the descending noradrenergic action on the $\alpha_1$-adrenoceptor, the histamine $H_2$ receptor-driven inhibitory action of GABAergic neurons was not strong enough to produce a significant antihypersensitivity effect. According to this hypothesis, blocking of the spinal $\alpha_1$-adrenoceptor alone was enough to attenuate the histamine-induced drive of the inhibitory GABAergic neuron below that needed to suppress pain-related signals.

The present study did not address the potential role of the histamine $H_4$ receptor that is expressed particularly on immunocompetent cells (Panula et al., 2015), but it is also present in the dorsal root ganglion neuron and the spinal dorsal horn (Strakhova et al., 2009). Since systemic administration of histamine $H_4$ receptor antagonist has reduced inflammatory and neuropathic pain hypersensitivity (Hsieh et al., 2010a; however, Sanna et al., 2015), it might be expected that histamine, due activation of the histamine $H_4$ receptor increases rather than decreases neuropathic hypersensitivity.

It may be concluded that spinal histamine at the dose of 10 μg selectively attenuates mechanical hypersensitivity and ongoing pain-like behavior in neuropathy. It is proposed that the antihypersensitivity effect induced by spinal histamine is at least partly mediated by histamine $H_2$ receptors and it is dependent on

co-existence of (presumably neuropathy-induced) $\alpha_1$-adrenoceptor-mediated drive of GABAergic neurons acting on the spinal $GABA_A$ receptor. Since central facilitation of hypersensitivity induced by NMDA was completely prevented by histamine, the spinal histamine-induced attenuation of responses in spinal pain-relay neurons was post- rather than presynaptic. The decrease in the magnitude of the antihypersensitivity effect with prolonged treatment suggests that spinal administration of histamine alone may not be a promising treatment for chronic neuropathy.

## Conflict of interest

The authors have recently received financial support for another histamine-related pain study from Abbvie Inc., North Chicago, IL, USA.

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