

Effect of Epinastine Hydrochloride, a Second-generation Histamine H₁-receptor Antagonist, on Sensory Neurons *in vitro*

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ABSTRACT

Background: Epinastine hydrochloride (epinastine) is a second-generation histamine H₁-receptor antagonist widely used as an anti-allergic and anti-pruritic. To explore possible new aspects of the anti-pruritic mechanism of epinastine, in particular any effects on the peripheral nervous system, we examined epinastine's effects on sensory neurons using cultured murine dorsal root ganglion (DRG).

Methods: We performed a quantitative assessment of neurite growth and substance P (SP) release from isolated DRG in the presence versus the absence of epinastine. Mechanism(s) of epinastine's effects on sensory neurons were detected by examining its neurotoxicity, inhibitory action on nerve growth factor (NGF), and modulatory function on NGF receptors.

Results: The percentage of DRG with outgrowing neurites, total number of neurites, and average extension length of neurites were decreased by epinastine in a concentration-dependent manner. Epinastine did not exhibit any evidence of neurotoxicity on sensory neurons, degradation and inactivation ability on NGF, or effects on expression of NGF receptors. Also, no effects on neural progenitor cells of the central nervous system in culture were observed. Epinastine suppressed capsaicin-induced SP release from DRG neurons in a dose-dependent fashion.

Conclusions: The results demonstrate that epinastine has inhibitory effects on sensory neuronal growth, which may explain its clinical effects including potent anti-pruritic activity.

KEY WORDS

epinastine, histamine H₁ antagonists, nerve growth factor, substance P

INTRODUCTION

Histamine H₁-receptor antagonists (antihistamines) are anti-allergic drugs used extensively for the management of various histamine-related allergic disorders such as atopic dermatitis, urticaria, and allergic rhinitis.¹ Mast cells are rich sources of many distinct biologically active multifunctional mediators including histamine, prostaglandins, leukotrienes, neutral proteases, cytokines, and neurogenic factors.² Histamine is a fundamental mediator released from tissue

mast cells during the immediate allergic response¹ and chiefly from recruited basophils during the late-phase response.³ Histamine interacts with H₁-receptors to induce smooth muscle contraction, enhanced capillary permeability, and neuronal stimulation with multiple secondary effects resulting in induction of neurogenic inflammation and onset of itch sensation in the skin.⁴ Antihistamines are hence the first-choice drugs for the treatment of cutaneous pruritic diseases such as atopic dermatitis in which mast cells and released histamine play significant roles.⁵

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Afferent somatic nerves with fine unmyelinated C-fibers derive from dorsal root ganglia (DRG) and innervate the skin. C-fibers can be activated by a wide range of physical and chemical stimuli, resulting in reception of cutaneous sensations such as itching, by intervention of the central nervous system.⁶ Neurogenic components are involved in the pathogenesis and onset of itch sensation of a variety of cutaneous inflammatory diseases via the neuroimmunocutaneous system.⁷ Increased attention has been directed towards interactions between components of the nervous system and multiple target cells of the immune system. Communication between nerves and mast cells is a prototypic demonstration of such neuroimmune interactions. Recent evidence suggests that substance P (SP), a neuropeptide belonging to the tachykinin family, is an important mediator in intimate nerve-mast cell cross-talk.⁸ There is also considerable evidence suggesting that the nervous system can influence the course of diseases through emotional stress, altered patterns of cutaneous innervation, and abnormal expression and increased levels of neuropeptide and neurotrophic factors such as SP and nerve growth factor (NGF) both in the lesional skin and peripheral blood.^{7,9-11}

Epinastine hydrochloride (epinastine) is a nonselective, second-generation histamine H₁-receptor antagonist commonly used in Japan with good clinical results.¹² In the dermatological field, epinastine has been shown to have clinically potent and long-lasting anti-pruritic effects with infrequent adverse reactions in patients even with a generalized, severe degree of pruritus such as in atopic dermatitis. Epinastine possesses inhibitory action on not only the H₁-receptor but also on inflammatory mediator release from mast cells.^{13,14} In addition to these common antihistamine activities, epinastine exerts a variety of unique pharmacological modes of action including modulating interleukin (IL)-4 mRNA expression by peripheral blood mononuclear cells,¹⁵ inhibiting IL-8 release from eosinophils,¹⁶ and preventing superoxide generation by neutrophils.¹⁷ To explore possible new aspects of epinastine's anti-pruritic mechanisms, especially direct effects on the peripheral nervous system, we examined its effects on sensory neurons using cultured murine DRG *in vitro*.

METHODS

ANIMALS AND DISSECTION OF DRG

ICR mice (Japan SLC, Shizuoka, Japan) of postnatal day 1 were used in the study. The experimental procedures for mice were conducted in accordance with the guidelines of the Guiding Principles for the Care and Use of Laboratory Animals approved by the Committee for Animal Experiments in Toyama Medical and Pharmaceutical University. All mice were anesthetized with an ip injection of 6 mg N-pentobarbital/100 g body weight. DRG from L1 to L6

were dissected and mice subsequently euthanized by CO₂ asphyxiation.

DRG CULTURE

DRG were desheathed in ice-cold DMEM (Gibco BRL, Carlsbad, CA, USA) supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 1% penicillin, streptomycin, Fungizone antibiotic solution (Sigma, St. Louis, MO, USA), and 10% heat-inactivated fetal bovine serum (Bioserum, Canterbury, Victoria, Australia) (basic medium [BM]). The tissue explants were then plated 6/well in a 12-well tissue culture plate (Becton Dickinson Labware, Lincoln Park, NJ, USA) coated with a thin layer of Matrigel (Collaborative Research, Bedford, MA, USA) in BM. Primary cultures of dissociated murine DRG neurons were prepared as described elsewhere.¹⁷ Both isolated DRG and dissociated DRG cells were incubated in BM at 37°C in humidified 5% CO₂/95% atmospheric air.

NEURAL PROGENITOR CELL AND PC12 CELL CULTURE

CC-2599 normal human neural progenitor cells (NPC) capable of differentiating into neurons and glial cells including astrocytes and oligodendrocytes¹⁸ were purchased from Clonetics (San Diego, CA, USA). NPC were cultured in exclusive serum-free medium (NPMM) containing 0.2% human recombinant basic fibroblast growth factor, 0.2% human recombinant epidermal growth factor, 2% neural survival factor, and 0.2% gentamicin and amphotericin-B (Clonetics). Approximately 2.5×10^4 cells/well were seeded onto a 24-well culture and placed in a 37°C humidified incubator with 5% CO₂ for 10 days. Rat pheochromocytoma PC12 cells purchased from American Type Culture Collection (Tokyo, Japan) were maintained in RPMI 1640 medium as described elsewhere.¹⁹ For proliferation and differentiation of PC12 cells, the cells were allowed to attach on the dishes before replacing the medium with RPMI 1640 supplemented with 2% fetal horse serum, NGF (1, 10, and 20 ng/mL) (Sigma), and epinastine at concentrations of 10^{-7} – 10^{-3} M.

NEURITE EXTENSION IN SENSORY NEURONAL CULTURES

Culture plates were immediately supplemented with NGF (1, 10, or 20 ng/mL) and epinastine at final concentrations of 10^{-9} – 10^{-3} M dissolved in BM. On the basis of our preliminary experiments, the percentage of DRG with outgrowing neurites was analyzed at 24 h and the number of DRG neurites after starting culture at 72 h. Twenty-four hours after exposure to epinastine, all DRG in culture were observed under a phase-contrast microscope (Nikon LWD 0.52, Tokyo, Japan) to assess neurite outgrowth from DRG. Processes longer than the explant diameter were scored

as positive. The number of neurites outgrowing from DRG was recorded by actual counting under a phase-contrast microscope 72 h after starting culture. To examine the effect of epinastine on extension length of neurites, DRG were cultured in BM with NGF for 12 h prior to treatment with the tested drug. Each culture well was then supplemented with 10^{-9} – 10^{-3} M epinastine into the culture medium. Phase-contrast photomicrographs of the whole area of each culture well were taken at 12-h intervals up to 72 h after starting culture. The total length of each neurite was measured with a computer-aided image analyzer system as described elsewhere.²⁰ Finally, the combined length and the number of neurites were calculated and divided by the number of DRG with neurites to yield the average extension length of neurites/12 h and the neurite number/DRG, respectively.

EFFECT OF EPINASTINE ON NEURITE EXTENSION IN NPC AND PC12 CELLS

Various concentrations of epinastine (10^{-7} – 10^{-1} M) were supplemented with NPC during culture for <10 days. The total number of neurospheroid colonies/well and morphology of neurospheres were comparatively evaluated in the presence versus the absence of epinastine. PC12 cells exposed to epinastine were visualized by phase-contrast microscope ($\times 100$ magnification). For quantification of neurite outgrowth, ≥ 100 single cells (not aggregated)/each area of 10 arbitrary positions on the dish were observed, and those with processes of length greater than the cell's diameter were counted as positive process-bearing cells. For the cell proliferation assay, cultured PC12 cells were detached by incubation for 5 min in 0.05% trypsin/0.02% EDTA, and the cell number was counted by a hemacytometer.

ASSESSMENT OF VIABILITY OF DRG CELLS

DRG cells were cultured in BM containing NGF and epinastine for 72 h, removed by trypsin, and the cell number was counted by a hemacytometer. To determine the effects of epinastine on DRG neuron survival, trypan blue exclusion was used as described previously.²¹

ENZYME-LINKED IMMUNOSORBENT ASSAY FOR NGF

NGF levels were measured in BM with or without epinastine by a highly sensitive, 2-site, immunoenzymatic assay as described elsewhere.¹¹ All procedures were performed using a commercial ELISA kit for NGF following a modification of the protocol described by Boehringer Mannheim (Germany). Samples composed of BM with NGF (10, 100, and 500 pg/mL) and epinastine (10^{-7} – 10^{-3} M) and standard solutions (0–1000 pg/mL) were used in the experiment.

INDIRECT IMMUNOFLUORESCENCE

Dissociated DRG cells were cultured in BM with NGF and 10^{-7} – 10^{-3} M epinastine for 72 h. Cells were harvested by 2 min incubation with 0.05% trypsin/0.02% EDTA and cell suspensions fixed with 2% paraformaldehyde solution. Cells were incubated with monoclonal antibody directed against p75 NGF-receptor (NGF-R) (1:50) (Upstate Biotechnology Inc., NY, USA) and polyclonal antibody against p140 *trk* NGF-R (1:50) (Santa Cruz Biotechnology, CA, USA) for 1 h at 37°C. Cells were then stained with phycoerythrin-conjugated anti-rabbit IgG1 (Southern Biotechnology Associates, Birmingham, AL, USA) for 20 min on ice. Cells were resuspended in cold phosphate-buffered saline and analyzed by flow cytometer (FACScan, Beckman Coulter KK, Tokyo, Japan). Mean fluorescence intensity/5000 DRG cells was measured electrically.

EFFECT OF EPINASTINE ON CAPSAICIN-INDUCED SP RELEASE FROM SENSORY NEURONS

DRG were cultured for 72 h in BM containing 10 ng/mL NGF and maintained in BM with 10^{-7} – 10^{-4} M epinastine. Six hours later, capsaicin (1, 10, or 100 ng/mL) (Sigma) was added to the culture medium. Supernatants were then collected and supplemented with 5% (v/v) protease inhibitor (Wako Pure Chemical Industries, Ltd., Osaka, Japan) to protect spontaneous degradation of SP. For determination of SP levels, highly specific radioimmunoassay for SP was performed, essentially as described previously.¹¹ To identify sensory neurons, cultured specimens were incubated with a primary polyclonal antibody against SP (Progen Immuno-Diagnostika, Heidelberg, Germany), a specific marker for DRG neurons, at a dilution of 1:200. Immunohistochemistry was performed by enhanced labeled polymer system (Dako, Kyoto, Japan) as described elsewhere.²² Cultured DRG neurons treated with epinastine were stained before and after exposure to capsaicin.

RESULTS

EFFECTS ON SENSORY NEURONAL GROWTH

Representative DRG neurons with outgrowing neurite processes in the absence and the presence of epinastine are shown in Figure 1. Less than 5% of DRG maintained in BM alone displayed neurite outgrowth, whereas 24 h after stimulation with 1, 10, and 20 ng/mL NGF, more than 70%, 80%, and 90% of DRG, respectively, exhibited outgrowing neurites. Twenty-four hours after exposure to epinastine, a clear concentration-dependent decrease in the percentage of DRG with outgrowing neurites was observed at $\geq 10^{-7}$ M epinastine (Fig. 2). The number of neurites from DRG was also significantly decreased by $\geq 10^{-7}$ M epinastine in a concentration-dependent manner (Fig. 3). The total length of neu-

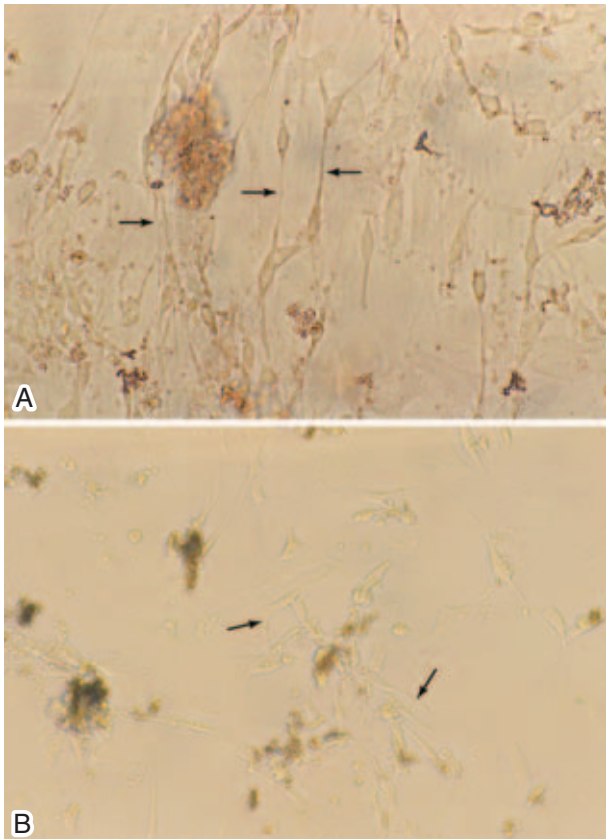


Fig. 1 Representative DRG neurons with outgrowing neurite processes (arrows) in the absence (A) and presence (B) of epinastine. DRG neurons of ICR mice were cultivated in BM containing 10 ng/mL NGF w/wo the addition of 10^{-6} M epinastine for 72 h. Note numerous neurites branching from DRG neurons in (A), which are greatly reduced in (B) by epinastine treatment. Original magnification, $\times 250$.

rites from DRG 72 h after starting culture significantly decreased by $\geq 10^{-6}$ M epinastine in BM with 1 and 10 ng/mL NGF and by $\geq 10^{-5}$ M epinastine in BM with 20 ng/mL of NGF (Fig. 4). While the average extension length of neurites/12 h without epinastine was relatively constant, epinastine suppressed the extension rates in a time-dependent fashion, starting from 12–24 h after supplementation, regardless of NGF concentration (Fig. 5).

NEUROTOXIC EFFECTS

The percentage of “dead” (*i.e.*, trypan blue-stained) DRG cells remained unchanged in all concentrations of epinastine added, ranging from 0.7–2% (data not shown). Furthermore, there was no significant difference in the percentage of viable cells in the absence versus the presence of epinastine.

NGF LEVELS

NGF levels measured by ELISA persisted unchanged

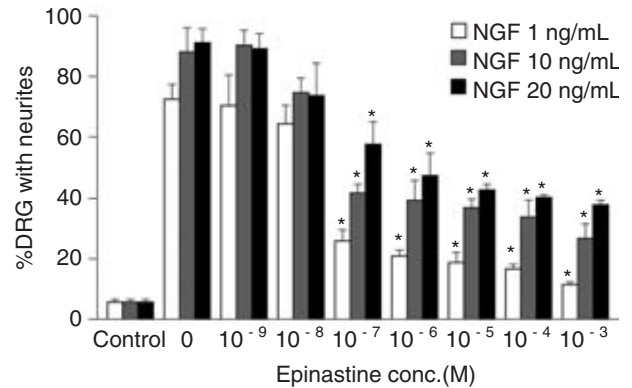


Fig. 2 Percentage of NGF-stimulated DRG with outgrowing neurites after 24-h exposure to epinastine at various concentrations. * $P < 0.05$

and no significant differences in NGF levels were found in the absence versus the presence of epinastine (data not shown).

INFLUENCE ON NEUROTROPHIC EFFECTS OF NGF ON PC12 CELLS

While PC12 cells neither proliferated nor extended neurite processes in the absence of NGF, cells bearing processes were observed in the presence of NGF. When increasing concentrations of epinastine from 10^{-7} – 10^{-3} M were added to PC12 cells cultured with NGF, no effects were observed in neurite extension, determined either by the percentage of process-bearing cells, or by the number of proliferating cells (data not shown).

EFFECTS ON NGF-R EXPRESSION

DRG cells expressed both p75 and p140 NGF-R. When DRG cells were exposed to epinastine 10^{-7} – 10^{-3} M, no significant difference in mean fluorescence intensity of either type NGF-R was observed at any concentration added (data not shown).

TOXICITY ON CENTRAL NERVOUS SYSTEM NPC

No visible morphological differences in neurospheroid formation and neurite extension were observed in the absence versus the presence of epinastine even at high concentrations. Furthermore, no significant difference in the number of colonies extending neurite processes was seen at any concentration of epinastine ≤ 10 days after culture starting from single cell suspensions (Fig. 6).

EFFECT ON CAPSAICIN-INDUCED SP RELEASE FROM DRG NEURONS

Baseline levels of SP released by capsaicin increased in a concentration-dependent manner. Epinastine $\geq 10^{-5}$ M suppressed SP levels in supernatants

Effect of Epinastine on Sensory Neurons

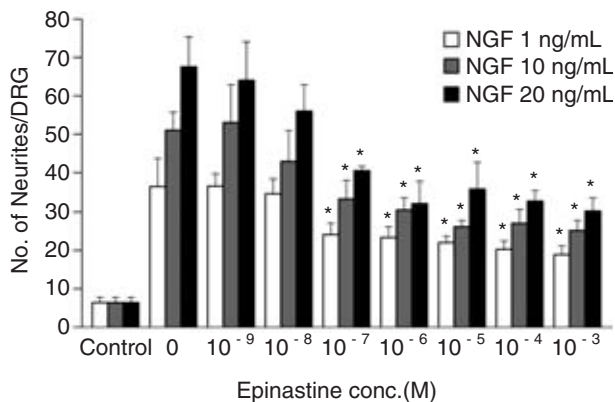


Fig. 3 Number of neurites from NGF-stimulated DRG after exposure to epinastine at various concentrations for 72 h. * $P < 0.05$

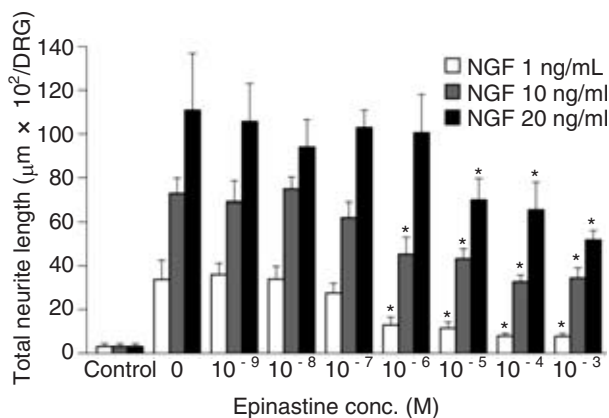


Fig. 4 Total length of neurites from NGF-stimulated DRG after exposure to epinastine at various concentrations for 72 h. * $P < 0.05$

after DRG neurons were exposed to capsaicin 1, 10, and 100 ng/mL in a concentration-dependent manner (Fig. 7). Cultured DRG neurons and extending nerve fibers showed intense immunoreactivity with SP, which diminished after exposure to capsaicin; however, epinastine-treated neurons remained positive for SP (data not shown).

DISCUSSION

The current study was conducted to determine whether epinastine has any direct influence on sensory neurons. Altered patterns of cutaneous innervation have been reported in many inflammatory dermatoses including atopic dermatitis^{9,10} and psoriasis.^{22,23} One major symptom of these skin disorders is pruritus, where sensory C fibers are stimulated both in the epidermis and in the superficial dermis. It has been reported that the epidermis and dermis of lesional skin from patients with atopic dermatitis and psoriasis with pruritus are significantly more densely

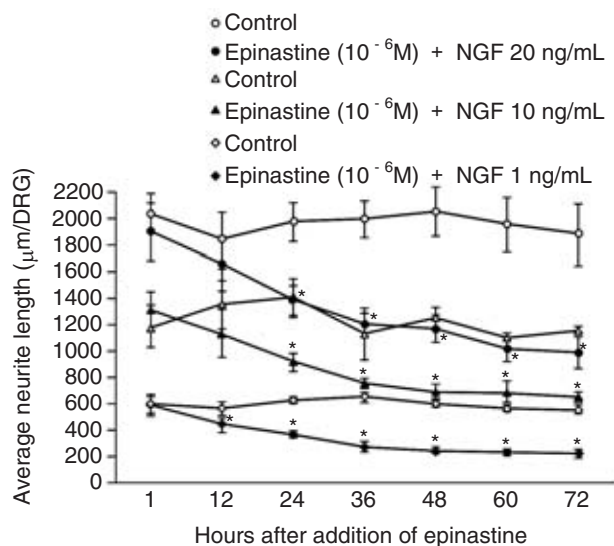


Fig. 5 Average extension length of NGF-stimulated neurites/12 h after addition of epinastine. * $P < 0.05$

innervated with nerve fibers than either lesion-free skin or normal healthy skin^{9,23} suggesting that patients with these pruritic skin disorders may be more vulnerable to intrinsic and extrinsic stimuli following stimulation of C fibers and that hyperinnervation in the skin of patients with pruritus may result in a lower itch threshold compared with in those without pruritus. In the present study, we observed epinastine concentration-dependent decreases in the percentage of DRG with outgrowing neurites, number of neurites/DRG, and average neurite extension length, indicative of novel anti-pruritic modes of action of epinastine. The concentrations of epinastine exerting inhibitory action on sensory neurons in the present study correspond roughly to those found in the blood *in vivo* after oral administration of the drug.²⁴ However, it is not yet certain whether such neuromodulatory effects are specific to epinastine among antihistamines. This will be the subject of further study.

NGF is the most fully characterized neurotrophic protein responsible for the development, differentiation, and survival of peripheral neurons. NGF guides neuritic growth in the periphery, thereby serving a trophic function,²⁵ which suggests that innervation in the skin resulting from neurite extension from DRG neurons may be modulated by NGF. NGF is transported in a retrograde fashion from target tissues and nerve terminals to neuronal cell bodies and exerts neurotrophic effects on some primary neural crest-derived sensory neurons and peripheral adrenergic and central cholinergic neurons.²⁶ Although the precise mechanism of epinastine's suppressive activity on sensory neurons is not clear, there remains the possibility that epinastine has direct neurotoxic effects on sensory neurons. Short-term exposure to

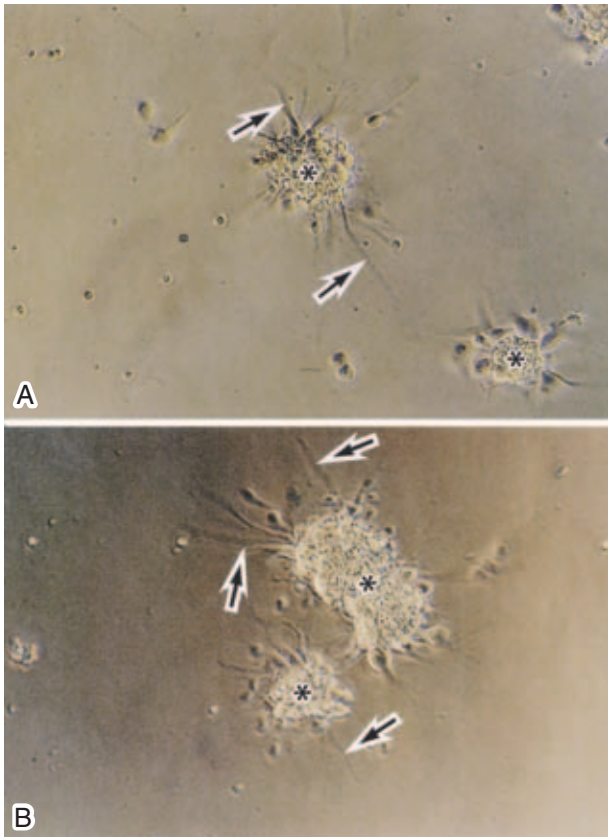


Fig. 6 Representative neurospheroids (asterisks) extending neurite processes (arrows) in the absence (A) and presence (B) of epinastine. Normal human neural progenitor cells were cultivated in the culture medium w/o the addition of 10^{-3} M epinastine for 10 days. No visible morphological differences in neurospheroid formation and neurite extension were seen between (A) and (B). Original magnification, $\times 420$.

some drugs such as lidocaine, a local anesthetic, has been reported to induce death in cultured DRG neurons *in vitro*.²⁷ Therefore we performed the present trypan blue exclusion experiments in dissociated DRG cells to observe cell viability after exposure to various concentrations of epinastine. As a result, the percentage of trypan blue-stained DRG cells remained unchanged at any concentration of epinastine added, and there was no statistically significant difference in the percentage of viable cells in the absence versus the presence of epinastine, suggesting that epinastine has no neurotoxicity. Another possibility regarding epinastine's inhibitory mechanism on sensory neurons is rapid degradation and/or inactivation of NGF. We measured NGF levels in BM containing low concentrations of exogenous NGF treated with epinastine by ELISA, and observed neurotrophic effects of NGF-containing medium supplemented with epinastine on rat pheochromocytoma PC 12 cells.

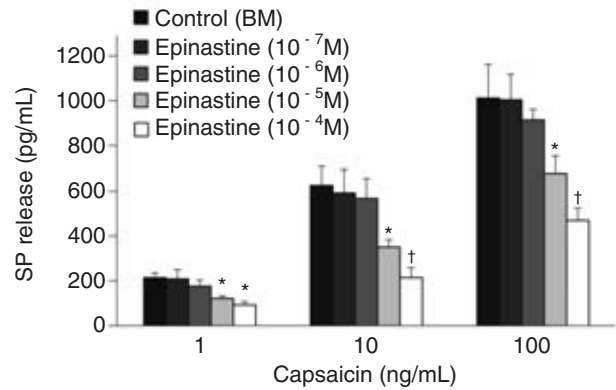


Fig. 7 Effect of various concentrations of epinastine on SP levels in supernatants after exposing DRG neurons to capsaicin. * $P < 0.05$; † $P < 0.01$

This approach was based on many previous studies on the intracellular signaling cascades for neurite outgrowth triggered by NGF which were performed in this cell line.²⁸ NGF levels in medium persisted unchanged after addition of epinastine and no significant differences in NGF levels were observed in the absence versus the presence of epinastine, suggesting that epinastine is unable to degrade NGF. Differentiation evaluated by the percentage of neurite process-bearing cells and proliferation measured by the number of PC 12 cells were uninfluenced by epinastine, further implying that epinastine may not inactivate NGF.

NGF exerts its effects by binding two classes of transmembrane receptors, a low affinity receptor ~ 75 kd (p75) and a high affinity receptor ~ 140 kd (trk A). While it has been demonstrated that expression of the trk A protooncogene is critical for NGF signal transduction in primary neurons,²⁹ much uncertainty exists concerning the functional role of the p75 receptor component. Both NGF-R subtypes have been identified on sensory nerves.³⁰ Increased levels of both p75 and trk A NGF-R mRNA have been demonstrated in DRG of rat following nerve injury.³¹ However, recent studies have shown that high-affinity binding of NGF to PC12 cells requires both the p75 and trk A NGF-R and that the relative numbers of each receptor determines the NGF binding affinity.³² Since the functional relationship between these NGF-R is still in dispute, we examined the effects of epinastine on both p75 and trk A NGF-R expression in DRG cells in culture by flow cytometry. No significant differences were found in mean fluorescence intensity of both p75 and trk A NGF-R at any epinastine concentration tested, indicating that the inhibitory activities of epinastine on sensory neurons may not be mediated by NGF-R. Hence the cellular mechanism for the suppressive effects of epinastine on sensory neurons remains unexplained.

We demonstrated that the central nervous system is uninfluenced by epinastine even when high concentrations of epinastine were added to cultured NPC. This may be extremely important because it became evident from our experiments that epinastine is not neurotoxic to multipotential progenitor cells in the central nervous system, suggesting that its inhibitory effects on neurons are specific against the peripheral nervous system. Taking into account epinastine's low penetration of the blood-brain barrier,³³ its effects on the central nervous system appear negligible. The underlying reason for the observed differences in response to epinastine observed in the peripheral and central nervous systems is not clear. Although NGF and NGF-R are localized to diverse brain areas and active glial growth is associated with expression of the NGF gene,³⁴ NGF's function during normal development and maturity remains to be elucidated. The expression of NGF by brain astrocytes is considered analogous to that of peripheral Schwann cells. While cultured Schwann cells contain high levels of NGF, the message is virtually undetectable in adult peripheral glia.³⁵ The sciatic nerve, which consists mostly of Schwann cells, expresses high levels of NGF mRNA at birth, with rapid decreases in expression during development. The NGF message is extremely low in the adult sciatic nerve.³⁶ We observed cultured NPC rapidly proliferating and differentiating into neural components of the central nervous system. Taken together, different contents of locally accumulated NGF derived from neuron-supporting cells may be associated with different responses of neurons to epinastine, via neuroprotective activity of NGF, between the central and peripheral nervous systems.

SP is associated with multiple cellular responses including vasodilatation, increased blood flow, plasma extravasation, mast cell degranulation, the wheal and flare reaction via axon reflex referred to as neurogenic inflammation, neutrophil and macrophage activation, and modulation of the release of proinflammatory cytokines and chemokines.⁷ Neurogenic inflammation is mediated by SP released from peripheral sensory nerve terminals, which in turn stimulates specific receptors on and releases histamine from mast cells; the released histamine then further excites other sensory neurons. When applied to the skin, SP elicits an itch sensation in human subjects.³⁷ SP is generally believed to elicit pruritus through the release of histamine from mast cells. In human subjects, H₁-receptor antagonists inhibit itch sensation induced by intradermal injection of low-dose SP.³⁷ Intradermal injection of SP elicits itch-associated response in normal as well as in mast cell-deficient mice.³⁸ Neurokinin-1 tachykinin receptors are involved in the itch-associated response induced by SP, but not in histamine release from mast cells.³⁸ These findings suggest that both mast cell-dependent and

independent mechanisms are involved in SP-induced itch-associated response.⁷ It is known that capsaicin enhances release of SP from sensory neurons and nerve terminals in both the central and peripheral nervous systems and induces tachyphylaxis after repeated application.³⁹ In the present study we demonstrate that capsaicin-induced SP release from cultured DRG neurons is inhibited by epinastine in a concentration-dependent manner. The immunohistochemical study, in which diminution of immunoreactivity of cultured DRG neurons and extending nerve fibers exposed to capsaicin was suppressed by pretreatment with epinastine, supports the assay experiment. Such inhibitory effects of epinastine on capsaicin-induced SP release from sensory neurons may, in part, explain epinastine's clinical efficacy against inflammatory pruritic skin diseases.

In conclusion, here we demonstrate for the first time that epinastine possesses pharmacological modes of action on cutaneous sensory neurons including suppressive effects on neurite extension and inhibitory effects on SP release. These functions may in turn contribute to the drug's potent clinical efficacy and marked relief of itch intensity in pruritic dermatoses such as atopic dermatitis. Better understanding of the mechanisms of these effects on sensory neurons should aid in the development of pharmacological strategies to establish effective usage of the drug against allergic skin diseases with pruritus. Further studies are necessary to examine the effect of other first/second-generation antihistamines to clarify whether this effect is specific for epinastine.

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