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Why do Nettles Sting? About Stinging Hairs Looking Simple but Acting Complex

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ABSTRACT

To cope with environmental stress, plants have developed various defensive mechanisms against prey. For this purpose, some plants use external structures, like mechanical thorns and toxic stinging hairs. Although four families (Urticaceae, Euphorbiaceae, Loasaceae, Hydrophyllaceae) have genera including stinging hairs, most studies on stinging hairs are focus on nettles (*Urtica* spp.) in the Urticaceae. A stinging hair consists of one stinging cell and surrounding pedestal cells. A number of chemicals have been proposed as the toxins that are introduced through nettle stings when in contact with human skin, such as acetylcholine, histamine and serotonin, with formic acid being the most common nettle toxin. The nettle sting might induce significant pain, stinging and wheal reactions that may last >12 hours. Recent studies supporting oxalic acid and tartaric acid as persistent pain-inducing toxins in nettle stings suggest that the toxins in the stinging hairs may be fairly complex. The mechanism of the stinging reaction is still far from being understood. This review summarizes some previous studies with additional assumptions in order to suggest some theories regarding synthesis, storage, and secretion of sting toxins. The immunological and some other physical responses after the skin is stung are also discussed. Although much of the knowledge from the stinging reaction is still fragmented and mysterious, the tiny structures and mechanisms of the stinging hairs are still amazingly interesting.

Keywords: formic acid, nettle-induced urticaria, pain-inducing toxin, *Urtica* spp.

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INTRODUCTION

Plants have to protect themselves from various environmental threats by their own systems, the most important one of which is the dermal system. The outermost layer of the dermal system, the epidermis, separates underlying tissues from the external environment; therefore, one of the important functions of the epidermis is to protect the underlying tissue from environmental stresses.

In most plants, epidermal cells can grow out to form uni- or multicellular appendages with various shapes, which are generally categorized as hairs or trichomes. One important function of hairs is to serve as protection against animal assaults. Although these protective functions are diverse among different plants, they can be grouped into two categories on the basis of mechanism. The first group involves mechanical defense through hair stings, while the second group have additional toxin release after hair stings, and its reaction depends mostly on their chemical effects (Haberlandt 1914).

The characteristics of mechanical protective hairs can be illustrated by their stiff bristles in certain angiosperms. The walls of the bristles are often calcified or silicified and covered with protuberances acting as barbs. One famous

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Invited Review

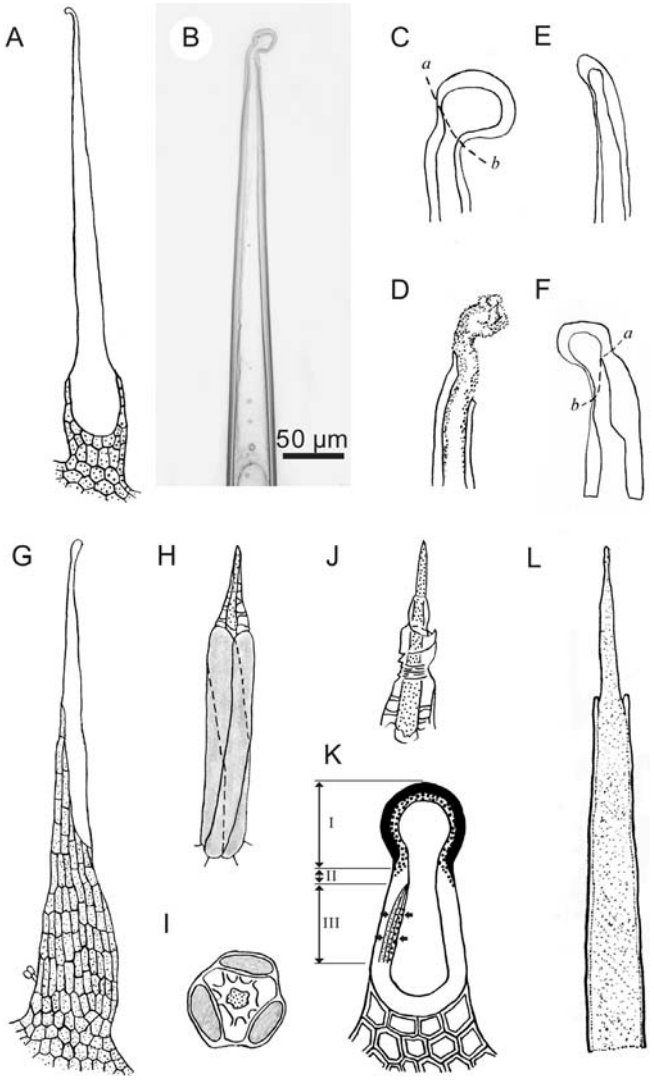

Fig. 1 Morphology of stinging hairs. (**A**) A stinging hair of *Urtica dioica* shows one elongated stinging cell with pedestal cells (dotted region) at base (Redrawn from Haberlandt 1914). (**B**) Light microscopy demonstrates upper region with intact bulb tip of stinging hairs of *U. thunbergiana*. Bar = 50 μm. (**C**)-(**D**) Apex of stinging hair of *U. dioica* with tip attached (**C**) and broken (**D**). The thinner wall at the narrow "neck" predefines the fracture line (a to b) (Redrawn from Haberlandt 1914). (**E**)-(**F**) Apical region of stinging hair of *Loasa papaverifolia* (**E**) and *Jatropha stimulata* (**F**) shows thicker wall at the convex side of the tip (Redrawn from Haberlandt 1914). (**G**) Stinging hair of *Girardinia cuspidata* shows extensive sheathing pedestal cells as dotted area (Redrawn and modified from Rouppert 1914). (**H**)-(**J**) Stinging hairs of *Dalechampia roezliana*. (**H**) Three twisted lateral cells (shaded region) are located under the central cell (Redrawn from Knoll 1905). Dotted area shows calcium oxalate crystal within the central cell. (**I**) One central cell containing calcium oxalate crystal (dotted area) and three lateral cells (shaded region) in cross section comprise the stinging hair. (**J**) Upon contact, calcium oxalate (dotted area) protrudes from the apical stinging cell. (**K**) Silica body distribution (shown as black spots and regions) of stinging cell wall of *U. pilulifera*. Silica bodies are mostly fused to a continuous network in region I, decreased in transition region II, and only found as secondary silica bodies (bold arrows) in region III (Redrawn and modified from Sowers and Thurston 1979). (**L**) Regeneration of cell membrane from a heavily broken tip of stinging hairs of *U. dioica* (Redrawn from Küster 1925).

genus of these groups is the cactus (*Opuntia*) that contains rather effective hook-like bristles with numerous barbs (Haberlandt 1914). Another example is rose prickles, which are typically sickle-shaped hooks. Those hairs would function as adaptation to reduce being browsed by mammals or being damaged by creeping animals.

The chemical protective hairs are generally called stinging hairs. The most famous plants containing stinging hairs are the *Urtica* spp., in which the toxic effect on human or other animals is frequently reported. After exposure in a field with *Urtica*, several dogs exhibited clinical signs included nausea, vomiting, clawing at the face and nose; the affected animals recovered within 36 hours after atropine and antihistamine treatment (Edwards and Remer 1983). Another report comes from misuse of *Urtica* leaves. Although extracts of *U. dioica* leaves may be used as herbal practice, directly chewing the leaves or sucking its sap would cause severe tongue oedema and pain lasting for at least five days (Caliskaner *et al*. 2004). Some field experiments demonstrate that the densities of stinging hairs were correlated with the herbivore damage. Herbivores preferentially grazed plants (*U. dioica*) with lower densities of stinging hair (Pollard and Briggs 1984). Moreover, the density of stinging hair increased in response to herbivore or mechanical damage (Pullin and Gilbert 1989). Since stinging hairs are quite effective at protecting plants from damage solely upon their tiny structures and trace amounts of toxins, many researchers were curious about their morphology and chemical components. This review provides an in-depth summary and discussion on recent progress of stinging hair structure and function.

STINGING HAIRS FUNCTION AS THE PROTECTIVE DERMAL STRUCTURE OF PLANTS

Stinging hairs are known in some genera of four families: Urticaceae, Euphorbiaceae, Loasaceae, and Hydrophyllaceae (for details, see **Table 1**) (Thurston and Lersten 1969). The general features of a stinging hair are one elongated, needle-like cell, called stinging cell, which can elicit irritants upon contact. Moreover, the neighboring cells, called pedestal cells or lateral cells, possess supportive functions at the base of the stinging cell. Due to different species having similar designs of stinging hairs to function as a chemical protective weapon, it provides a good example of convergent evolution among those species (Fu *et al*. 2003).

Morphology of stinging hairs

Regardless of the difference in size and chemical composition, the stinging hairs could be roughly classified into two types by morphology and sting mechanism. The first type, known in *Urtica* and most genera, was already noted and described by Robert Hooke in 1665. Each stinging hair usually contains one tapered, elongated stinging cell with a bulbous base embedded in sheathing pedestal cells (**Fig. 1A**). Upon contact, the tip breaks off and converts the stinging cell into a tiny hypodermic needle capable of penetrating the skin and injecting its constituents (Thurston and Lersten 1969). In apical regions of the stinging cells, the unequal thickness of cell walls makes tips easily broken. In the case of *Urtica*, both convex and concave sides of the neck, just below the oblique symmetrical bulb tip, contain relatively thin cell walls compared with neighboring regions (**Fig. 1B-D**). The bulb is first differentiated at the beginning of the sting ontogeny, prior to pedestal cell differentiation (Thurston 1974). This arrangement not only facilitates the detachment of the tip, but also forms a predefined fracture line, converting the tip to lancent-shape easily penetrating into the skin (Haberlandt 1914). A similar design could be also seen in *Laportea*, *Girardinia*, and *Dendrocnide* in Urticaceae (Thurston and Lersten 1969; Fu *et al*. 2003). Although some genera, like *Loasa* and *Jatropha*, have thin walls at concave sides but relatively thick walls at convex sides of the tip (**Fig. 1E, 1F**), the fracture line is still pre-formed (Haberlandt 1914). In some stinging hairs of *Wigandia* and *Loasa*, symmetrical bulb tips without thin-walled necks can be seen, but the rupture mechanism is not known (Thurston and Lersten 1969).

Besides the cell wall design of the stinging hair tip, the pedestal cells cover the stinging hair with different extents among different genera. In *Urtica thunbergiana*, the pedestal cells emerge from the epidermis, then cover and raise

Table 1 Plants with stinging hairs (After Thurston and Lersten 1969; Lovell 1993).

Family	Genera
Urticaceae	*Fleurya*, *Dendrocnide*, *Girardinia*, *Gyrotaenia*, *Hesperocnide*, *Laportea*, *Nanocnide*, *Obetia*, *Scepocarpus*, *Urera*, *Urtica*
Euphorbiaceae	*Acidoton*, *Caperonia*, *Cnesmone*, *Cnidoscolus*, *Dalechampia*, *Jatropha*, *Megistostigma*, *Pachystylidium*, *Platygyna*, *Sphaerostylis*, *Tragia*
Loasaceae	*Blumenbachia*, *Caiophora*, *Cevillavia*, *Eucnide*, *Fuertesia*, *Gronovia*, *Loasa*
Hydrophyllaceae	*Wigandia*

half the bulbous base of the stinging cell (~2.7 mm in length) (**Fig. 1A**). The tiny stinging cell in *Dendrocnide meyeniana*, about 0.4 mm long, is surrounded by one circle of pedestal cells at the base without obvious emergence, while more than half the length of the stinging cell (total length ~5.7 mm) is sheltered by pedestal cells in *Girardinia diversifolia* (Haberlandt 1914; Rouppert 1914; Fu *et al*. 2003) (**Fig. 1G**).

Another type of stinging hair consists of one central stinging cell with calcium oxalate crystals in its apical regions, enclosed by a "cellulose cover" with uneven thickness in *Tragia* and *Dalechampia* (Knoll 1905; Thurston 1976) (**Fig. 1H**). The crystal is attached by "cellulose beams" to the outer wall of the central cell (Thurston 1976). This crystal is ejected from the cell upon contact and punctures the skin, mechanically producing an initial stinging sensation; a secondary reaction occurs when other contents in the crystal enter the wound (Knoll 1905; Thurston and Lersten 1969) (**Fig. 1J**). Three or four lateral cells completely surround the central stinging cell (**Fig. 1H, 1I**), but possess no plasmodesmata or pits connecting to the stinging cell unlike pedestal cells in *Urtica* and *Cnidoscolus* (Thurston 1974, 1976).

Both types of stinging hairs have dual origins, from the epidermis and hypodermis, but with some differences. The stinging cell in *Urtica* and *Loasa* is stemmed from the protrusion of one epidermal cell, with mature pedestal cells from numerous cell divisions in both the epidermal and subepidermal cells (Thurston and Lersten 1969; Thurston 1974). However in *Tragia* and *Dalechampia*, the stinging hair originates by a protrusion of a single subepidermal cell between 3-4 adjacent epidermal cells. Because of their dual origins, the stinging hairs should strictly be regarded as emergences, different from typical trichomes, defined as outgrowths from the epidermis only (Thurston 1974, 1976).

What is a "nettle"?

Although stinging hairs are present in different species, the most famous and extensively studied plants with stinging hairs are *Urtica* spp., also termed "nettles". It should be noted, however, that this common name "nettle" can also be generally interpreted as all kinds of plants bearing stinging hairs (Lookadoo and Pollard 1991). Some stinging plants have been given a similar name with "nettle," namely, "tree nettle" for *Laportea* or "spurge nettle" for *Cnidoscolus*.

One serious problem concerns the consequent identification responsible for the stinging reaction induced by toxic plants. For example, although it has been reported that the sting of the "nettle" has caused death in hunting dogs, it is doubtful whether death as the extreme sign is caused by massive stinging hairs of *Urtica*, or involved in other contacts like invertebrates or heavy pollen (Edom 2002). Before studying the toxic mechanisms and functions of the stinging hairs, the species and the characteristics of its stinging hairs should be examined to illustrate the phenomenon of the sting reactions.

TOXINS OF STINGING HAIRS

Contact of human skin with stinging hairs can induce non-allergic contact urticaria, or nettle-induced urticaria, in which the irritant is released and produces wheals and flares with a pain or stinging sensation that may last for more than 12 hours (Oliver *et al*. 1991; Taskila *et al*. 2000). Toxin identification of sting hairs has had a long history with many identified toxins. Though Thurston and Lersten (1969) provided a nice review on toxicology of plant stinging hairs, no updated reviews have been published in about four decades. Since some earlier announced toxins are still disputed and new toxins were discovered in recent years, this review provides an updated discussion on those toxins. A complete list of possible toxic chemicals in stinging hairs are given in **Table 2** (modified from Thurston and Lersten (1969) with new references added). The following discussions will focus on chemicals which are still debated or found in the last 20 years, since the older contentions of the remaining chemicals can be found in Thurston and Lersten's review (1969). **Table 3** summarizes references used in the following discussion, including studied species, methods, and major results or purposes.

Table 2 Possible toxic constituents reported in stinging hairs (listed by genus and chronologically).

Genus	Possible toxic constituents	References
Urtica	Salt	Hooke 1665
	Formic acid	Gorup-Besanez 1849; Bergmann 1882; Dragendorf 1905; Winteritz 1907; Flury 1927; Nestler 1925
	Glucoside	Rauter 1872; Kroeber 1928; Starkenstein and Wasserstrom 1933
	Enzyme	Haberlandt 1886; Nestler 1925
	Tartaric acid	Gibson and Warham 1890; Fu *et al*. 2006
	Alkaloids	Giustiniani 1896; Winteritz 1907; Starkenstein and Wasserstrom 1933
	Calcium	Winteritz 1907
	Resin acid	Flury 1927
	Acetylcholine	Emmelin and Feldberg 1947, 1948; Collier and Chesher 1956; Saxena *et al*. 1965; Marty 1968; Maitai *et al*. 1980; Corsi 1992
	Histamine	Emmelin and Feldberg 1947, 1948; Collier and Chesher 1956; Saxena *et al*. 1965; Maitai *et al*. 1980; Czarnetzki *et al*. 1990; Oliver *et al*. 1991; Taskila *et al*. 2000; Fu *et al*. 2006
	Serotonin (5-hydroxytryptamine)	Collier and Chesher 1956; Saxena *et al*. 1965; Regula and Devidé 1980; Oliver *et al*. 1991
	Calcium-sensitive factor	Pilgrim 1959
	Leukotrienes	Czarnetzki *et al*. 1990
	Platelet-activating factor	Antonopoulou *et al*. 1996
	Oxalic acid	Fu *et al*. 2006
Loasa	Acetic acid	Tassi 1886
Tragia	Formic acid	Ritterhausen 1892
	Protein	Knoll 1905
Laportea	Formic acid	Petrie 1906
	Acetic acid	Petrie 1906
	Resin acid	Flury 1927
Girardinia	Acetylcholine	Saxena *et al*. 1966
	Histamine	Saxena *et al*. 1966
	Serotonin (5-hydroxytryptamine)	Saxena *et al*. 1966
Cnidoscolus	Serotonin (5-hydroxytryptamine)	Lookadoo and Pollard 1991

Table 3 Brief summary of chemical identification and related topic concerning the toxic constitutes in stinging hairs. See text for detail discussions.

Year	Authors	Species	Major methods	Major results and comments
1947	Emmelin and Feldberg	*Urtica urens*	Pharm., human skin pricking test	*Ac: 0.1 μg/stalk hair, 0.053 μg/leaf hair; *Hi: 0.011 μg/stalk hair, 0.005 μg/leaf hair.
1948	Emmelin and Feldberg	*Urtica dioica*	Pharm.	Whole plant contains Ac and Hi.
1956	Collier and Chesher	*Urtica dioica*	Pharm.	Ac, Hi, and *Se exist.
1957	Robertson and MacFarlane	*Laportea moroides*	Pharm., injection of human skin	Ac, Hi, and Se –like activity exists, but a non-dialysable substance may be the essential pain-producing materials.
1963	Macfarlane	*Laportea*	Pharm., injection of human skin	Not Ac, Hi, or Se because toxins are stable on drying (46 years) and boiling (10 min).
1965	Saxena *et al*.	*Urtica parviflora*	Pharm., PC	Ac: 318.4 μg/g leaf; Hi: 38.8 μg/g leaf; Se: 0.25 μg/g leaf.
1966	Saxena *et al*.	*Girardinia heterophylla*	Pharm., PC	Ac: 40 μg/g leaf; Hi: 3.75 μg/g leaf; Se: 0.15 μg /g leaf.
1968	Marty	*Urtica urens*	Histochemistry, microscopy	Ac occurs in vacuolar system of the sting.
1970	Oelrichs and Robertson	*Laportea moroides*	Chromatography, pharm.	Two pain-producing agents of high molecular weight are reported yet not completely identified.
1980	Maitai *et al*.	*Urtica massaica*	Pharm.	Ac: 5.2 μg/g hairs; Hi: 550 μg/g hairs.
1980	Regula and Devidé	Several *Urtica spp*.	Chromatography, histochemistry	Se exists.
1988	Kulze and Greaves	*Urtica dioica, Urtica urens*		Question about persistant paraesthesiae, or other neurotoxic factor than Ac, Hi, or Se.
1990	Czarnetzki *et al*.	*Urtica urens*	Spectrofluorometry, radioimmunoassay , RP-HPLC, neutrophil chemotaxis assay	*Leukotrienes (LTB_4/LTC_4/LTD_4).
1991	Oliver *et al*.	*Urtica dioica*	Histology, ultrastructure	Application of stinging hairs to the skins activated mast cells. Hi: 6.1 ng/hair; Se: 33.25 pg/hair.
1991	Lookadoo and Pollard	*Cnidoscolus texanus*	Thin-layer chromatography, Spectrophotometry, RP-HPLC	Ac and Hi did not exist, but Se existed.
1992	Corsi	*Urtica membranacea*	Histochemistry	Ac and Se occurred in both stinging cells and pedestal cells; Hi in glandular trichomes.
1996	Antonopoulou *et al*.	*Urtica dioica*	Analytical chromatography	*Platelet-activating factor.
2000	Taskila *et al*.	*Urtica dioica*	Microdialysis	Low concentration of histamine and no LTC_4 released in human skin.
2006	Fu *et al*.	*Urtica thunbergiana*	RP-HPLC, pain behavioral assay	*Oxalic acid and tartaric acid causes major persistent pain.

Abbreviations: Pharm., pharmacological technique; PC, paper chromatography; RP-HPLC, reversed phase-high performance liquid chromatography; Ac, Hi, Se: acetylcholine, histamine, and serotonin, respectively. Concentration or location of toxins are showed, if reported. * indicates new chemicals identified.

Formic acid

Formic acid is the most debated toxin in the stinging hairs. It was first described as a stinging hair toxin in *Urtica* by Gorup-Besanez (1849), but doubted later by Haberlandt (1886). The argument was continued until Flury (1927), who concluded that the toxins in *U. dioica* were not formic acid, an enzyme, or a toxalbumin. Two prominent evidences showed that formic acid is not the major toxin in *Urtica*. First, it is estimated that only about 0.06 ng formic acid exists per sting hair in *U. urens* (Dragendorf 1905), equal to 0.0006% if the fluid volume is taken as 10 nL. However, over 5% formic acid was needed to produce a distinguished sensation in the skin prick test (Emmelin and Feldberg 1947). Our recent studies in *U. thunbergiana* also showed that formic acid in the stinging hairs was a minor ingredient, while even 10% formic acid could not induce persistent pain as stinging hairs applied (Fu *et al*. 2006). It is curious that formic acid is still regarded as a common toxin in the stinging hairs, while almost all studies suggested that formic acid is unlikely to play a role in the stinging reaction (Kulze and Greaves 1988; Edom 2002).

Acetylcholine, histamine, and serotonin (5-hydroxytryptamine)

Emmelin and Feldberg (1947) were the first group to investigate toxins by pharmacological bioassays, namely, through comparing the effect of hair fluid extract and known chemicals on muscle contraction or blood pressure. They identified three smooth muscle contraction-inducing substances in the stinging hairs of *U. urens*: acetylcholine, histamine, and an unidentified one. The prominent evidence for the presence of histamine and acetylcholine was that the burning and itching sensation elicited by histamine plus acetylcholine resemble that produced by the stinging hairs in the human prick test. Later studies from Collier and Chesher (1956) in *U. dioica* suggested that the third smooth muscle contraction-inducing substance was serotonin. Moreover, Collier and Chesher's studies further showed another unidentified pain-inducing substance.

Concentrations of acetylcholine, histamine and serotonin have been determined from different parts of plants and various species based on similar pharmacological bioassays. In the stinging hairs, the concentration of acetylcholine and histamine ranged from 0.03-0.16 μg/hair (about 1%) and 0.005-0.022 μg/hair (about 0.1%) respectively in *U. urens* and it was 0.013-0.067 μg/hair and 0.0035-0.01 μg/hair respectively in *U. dioica* (Emmelin and Feldberg 1947, 1948). Both acetylcholine and histamine showed lower concentrations in leaf and stem tissues (Emmelin and Feldberg 1948). The concentration of serotonin was the lowest, ranging between 3.43 and 4.86 ng per sting in *U. dioica* (Collier and Chesher 1956). All the three chemicals were also reported from whole leaf extracts of *U. parviflora* and *Girardinia heterophylla* (Saxena *et al*. 1965, 1966).

Although these three chemicals were identified as toxins in the stinging hair, they did not seem to exist in all

species with stinging hairs. For instance, serotonin did not occur in *U. urens* (Regula and Devidé 1980), or in *U. massaica* (Maitai *et al*. 1980). In contrast, serotonin, but not acetylcholine and histamine, was found in the sting of *Cnidoscolus texanus* (Euphorbiaceae) (Lookadoo and Pollard 1991).

While these three chemicals were regarded as toxins, they were debated from their first identification. In a prick test from Emmelin and Feldberg's study (1947), acetylcholine and histamine treatment only induced pain sensation in the first 2 minutes that could not explain the persistent tingling paraesthesia by nettle sting (Kulze and Greaves 1988; Oliver *et al*. 1991). Our recent studies also showed that even at high concentrations (10%), acetylcholine or histamine alone could not induce significant pain in rat behavioral assays (Fu *et al*. 2006). Although 2% serotonin could produce persistent pain, serotonin was undetectable in the fluids from sting hair of *U. thunbergiana*, indicating that serotonin may not be a major pain inducing agent in the sting hair (Fu *et al*. 2006). Moreover, microdialysis assay showed that only weak release of histamine (<100 nM) after being stung and to obtain flare and wheal reaction, 150-500 nM and 1500-5000 nM histamine, respectively, are required (Petersen *et al*. 1997; Taskila *et al*. 2000). In *Dendrocnide* (*Laportea*) *moroides* (Urticaceae), intradermal injection of dialyzed sting extracts, free of acetylcholine, histamine, and serotonin, produced the same sensation as that produced by untreated sting extracts. Taken together, these results suggest that the persistent pain-inducing agents, at least in *Dendrocnide*, do not include acetylcholine, histamine, and/or serotonin, but a stable, non-dialyzable, yet unidentified toxin (Robertson and MacFarlane 1957; Macfarlane 1963; Oelrichs and Robertson 1970).

Leukotrienes and platelet-activating factor

In an attempt to identify chemicals that produce persistent contact urticaria produced by stinging hairs, some mediators originally recognized in the inflammatory skins were surprisingly found in the stinging hairs as well. The first compound belonging to this category was leukotriene (LT), including leukotriene B_4 (LTB_4) and leukotriene C_4/D_4 (LTC_4/LTD_4) at concentrations of 0.15 pg and 0.3 pg per hair, respectively in *U. urens* (Czarnetzki *et al*. 1990). Moreover, platelet-activating factor (PAF) was also proposed as a toxin from whole nettle extracts of *U. dioica*, yet no quantitative data was available (Antonopoulou *et al*. 1996).

Some clinical studies suggested these chemicals might resemble the reaction induced by stinging hairs, although this coincidence is not satisfied. In humans, intradermally injected LTC_4 or LTD_4 (both 1.0 nmol, equivalent to ~625 and 500 ng, respectively) resulted in a persistent wheal in 2 hr and flare in 6 hr. With a higher dosage of LTB_4 (1.6 nmol, ~540 ng) injection elicited a transient wheal and flare, followed in 3-4 hr by induration characterized by a dermal infiltration comprised predominantly of neutrophil (Soter *et al*. 1983). However, neutrophils did not increase significantly in skin after being stung (Oliver *et al*. 1991). Furthermore, the amounts of leukotrienes in the stinging hairs (<1 pg) were significantly lower than that for injection (~500 ng), although lesser amounts (0.01-10 ng) of LTC_4 and LTD_4 could produce a wheal in 30 min (Juhlin and Hammerström 1983). No release of LTC_4 in nettle-induced urticaria by microdialysis provided negative evidence as well (Taskila *et al*. 2000).

Results from injected PAF (30-300 ng) also presented wheals and flares after 15 and 5 min, respectively (Sciberras *et al*. 1987). However, since PAF was found from whole nettle extracts (Antonopoulou *et al*. 1996), the existence of PAF in the stinging hairs deserves further verification.

Oxalic and tartaric acids

In addition to wheals and flares, another index of nettle-induced urticaria is persistent pain or stinging sensation. On the basis of rat behavioral pain tests, oxalic and tartaric acids were found as the major persistent pain-inducing toxins in the stinging hairs of *U. thunbergiana* (Fu *et al*. 2006). However, to obtain similar levels of reactions applied by stings, the amounts of intradermally injected oxalic and tartaric acids were 400 and 2000 μg, respectively, while much less oxalic (4.8 ng) and tartaric acid (57.2 ng) were found from one stinging hair. More delicate experiments to link the stinging reaction with human responses would be plausible to further clarify oxalic and tartaric acids as major persistent pain-inducing toxins in clinical cases (Oliver *et al*. 1991; Taskila *et al*. 2000).

MECHANISM OF STINGING REACTION: PLANT'S VIEW

Although most recent studies on stinging hairs focused on toxin identification, the process of toxin synthesis and storage has received little attention. Since the stinging reactions are a complicated combination between the toxins and the structure of the stinging hair, the structural advantage should be concerned with mechanisms of the stinging reaction. Indeed, some studies indicate that the special structural components of the stinging hairs in *Urtica* are important for transporting toxins and enhancing stinging functions (Haberlandt 1914; Marty and Buvat 1968; Fodor and Cseh 1993). Therefore, in an attempt to depict the draft of the stinging reactions, the proposed pathways of toxin synthesis, as well as release and its related mechanisms will be addressed and discussed.

Toxin synthesis pathway

Acetycholine, histamine, and serotonin are well-known neurotransmitters in animals, and their synthesis pathways in animals are well-known. In plants, these three chemicals also bear physiological functions other than toxins; therefore, their synthesis pathways in plants have also been studied (Roshchina 2001). The best characterized toxin synthesis pathway in the stinging hairs is that of acetylcholine. Acetylcholine is synthesized from two precursors, acetyl-CoA and choline, by choline acetyltransferase (**Fig. 2A**) (Roshchina 2001). Choline acetyltransferase, indeed, was detectable in the leaves of the nettle *U. dioica* and its enzyme activity was lower at more expanded mature leaves compared with young leaves. This reduced activity may be correlated with lower sting density in the fully expanded leaves (Barlow and Dixon 1973). Serotonin synthesis is regarded as detoxifying ammonium from tryptophan, with two alternative pathways, one mediated by 5-oxytryptophan, and the other, tryptamine (**Fig. 2B**) (Roshchina 2001). Although enzymes related to serotonin synthesis have been found in some plants, they have not been identified in plants with stinging hairs. Histamine is formed directly from histidine by histidine decarboxylase in animal tissues (Roshchina 2001); however, little was known regarding its synthesis pathway in plants.

Tartaric acid and oxalic acid are common ingredients in plants, while detailed studies on their synthesis pathways are still in progress. Interestingly, they share a common precursor, ascorbic acid. Two synthesis pathways from ascorbic acid to tartaric acid have been proposed (for details, see Loewus 1999). One pathway is shared with oxalic acid synthesis in oxalate-accumulating plants. That is, ascorbic acid undergoes a C2/C3 cleavage into oxalic acid and threonic acid, and then the latter is further oxidized to tartaric acid (**Fig. 2C**; Loewus 1999). Since many plants with stinging hairs contain calcium oxalate crystals in the leaves (Fu *et al*. 2003), it is likely that the synthesis of oxalic and tartaric acids utilizes this pathway.

In regard to other mentioned toxins, leukotrienes and

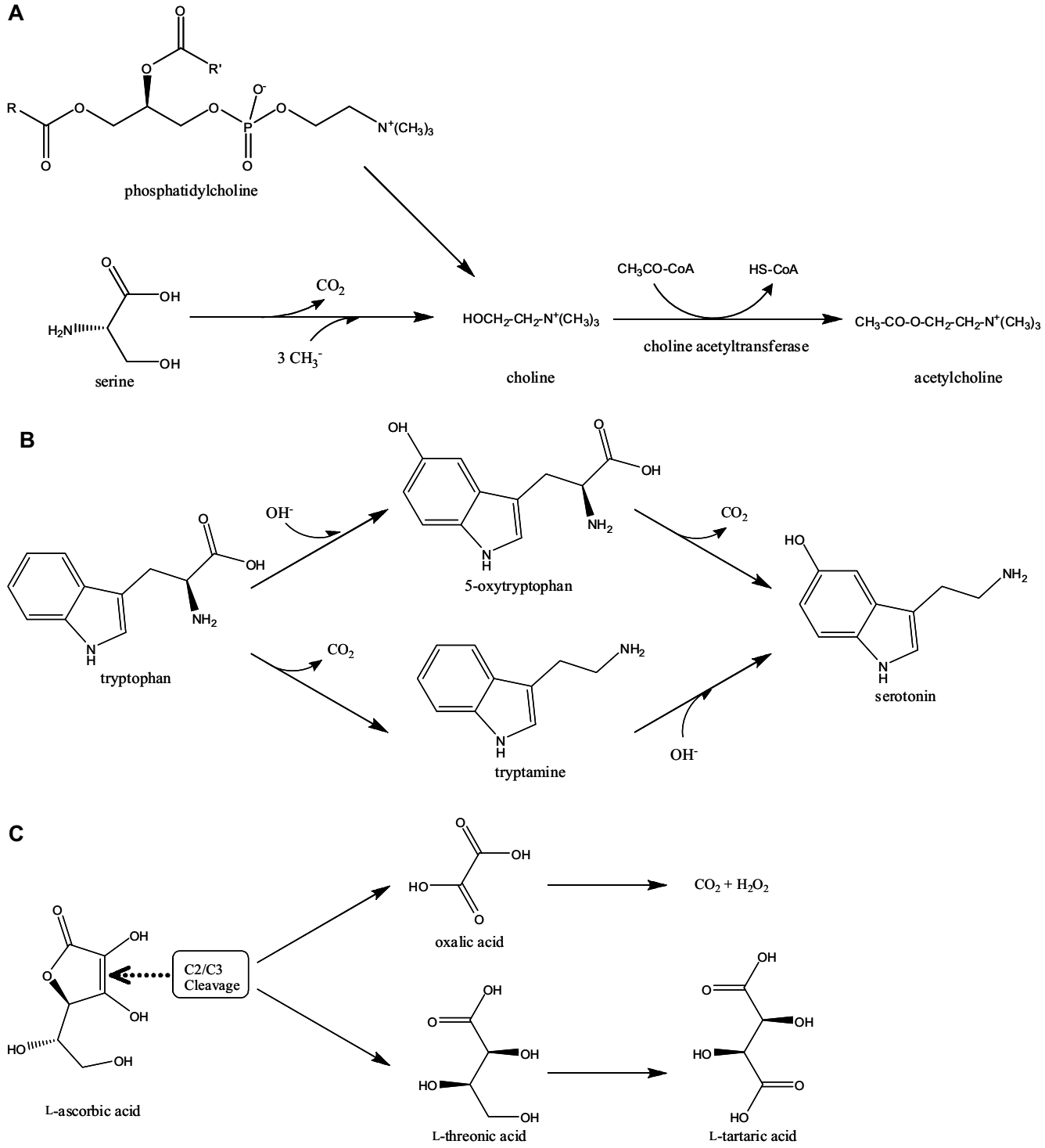

Fig. 2 Chemical synthesis pathways of acetylcholine (A), serotonin (B), oxalic acid and tartaric acid (C) in biological systems. See text for details. Adapted from Loewus 1999; Roshchina 2001.

platelet-activating factors, their synthesis pathways are well established in animals (Hammarström 1983; Prescott *et al*. 1990); however, no information regarding their synthesis in plants is available.

Toxin source and storage

Most discussion of toxin synthesis, transporting and storage are derived from the studies of stinging hairs in *Urtica*. Previous discussion implied that the toxins were synthesized in the pedestal cells and subsequently entered into the stinging cell, as evidenced by observation of conspicuous simple pit pairs in the wall between the bulbous basal region of the stinging cell and adjacent pedestal cells (Rauter 1872; Haberlandt 1886). However, a later study did not find such pits in developing or mature stinging cells under light or electron microscopy (Thurston 1974). Some other studies also implied that the toxins are synthesized outside of the stinging cell according to the observation that acetylcholine and histamine were detected in both stinging hairs and leaf tissues (Emmelin and Feldberg 1947, 1948) and that serotonin was detectable in both stinging cells and pedestal cells (Corsi 1992). However, those observations could not exclude the alternative hypothesis that the stinging cells individually produce toxins, independent of other cells.

No matter where the toxins are synthesized, it is plausible that the toxins will store at the upper regions of the stinging cells to facilitate their immediate release into the penetrating tissue. Cytochemical studies showed that the transportation of acetylcholine from the basal region to the

top of the sting might be mediated by vacuolar systems like the endoplasmic reticulum and the Golgi apparatus; namely, small vacuoles may contain acetylcholine and secrete to the cell wall by incorporation of plasma membranes (exocytosis) (Marty and Buvat 1968). Highly active cyclosis of the stinging cell (Wiesner 1906; Fu *et al*. 2003) may provide a mechanism for these vacuoles to circulate with cytoplasmic streaming.

Stinging in action: hair breakage, toxin secretion, and hair regeneration

Stinging cells provide at least two structural advantages for breaking their tip, and hence secret toxins. One is the predefined fracture line on the tip as mentioned above, and another is the silicification of the stinging cell. Primary silica bodies are found throughout the apex region of the cell walls and decrease basipetally. At basal regions, cell walls are either calcified with few secondary silica bodies in *Urtica*, or strongly lignified in *Jatropha* (Haberlandt 1914; Sowers and Thurston 1979) (**Fig. 1K**). When *U. dioica* were cultured in Si-deficient conditions, stinging cells lost their rigidity and the tip could not break off, and consequently, the stinging effect did not function as usual (Fodor and Cseh 1993). The stinging ability can be recovered by culturing in water containing SiO_2 solution for two weeks (Barber and Shone 1966). Altogether, the architecture of the silicified cell wall not only provides the stiff and brittle feature (Haberlandt 1914), but also ensures that the apex region is the most vulnerable site for stinging cell breakage.

One may suggest that the needle-like structure of the sting had additive effects of stinging sensation upon contact. However, repeated stroking with dried stinging hairs did not cause a stinging sensation, but a short-lived pain may occur due to mechanical puncturing of the skin (Maitai *et al*. 1980). Furthermore, while regular scaled needles (such as syringes) may deliver noticeable pain, microneedles with much smaller size (hundreds of μm^2 tip area) will only elicit light skin irritation (Mikszta *et al*. 2002). Since the tip diameter of the stinging hair is ~10 μm in *Urtica*, short contact with the stinging hair may only elicit unnoticeable, or very mild and temporary mechanical pains. Therefore, an occasional touch with the stinging hair hardly causes mechanical pain by its needle-like structure. Instead, the chemicals within the sting hair are the actual cause of the persistent pain.

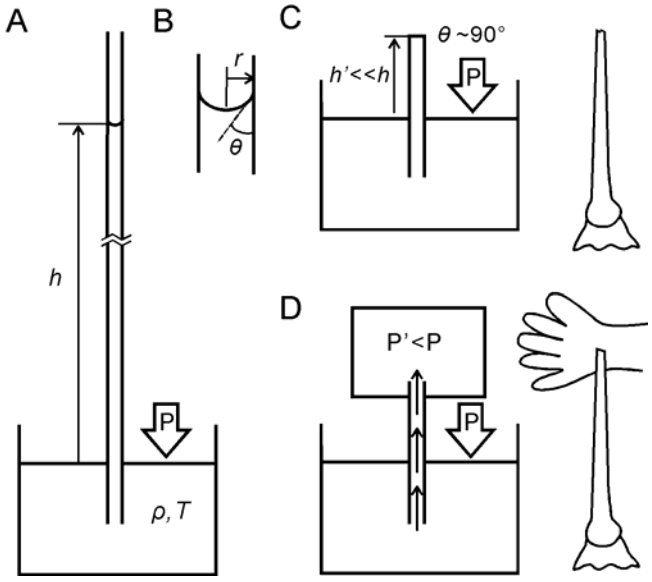

Fig. 3 Hypothetical stinging mechanism considering the capillary force and pressure difference. (**A**) Placing a long thin tube in a container causes inside liquid pull up along the tube wall by capillary force. (**B**) Enlarging views of liquid surface in the tube. Denotations of (**A**) and (**B**) are used in text. While a stinging hair is broken, it will not exude its fluids due to capillarity (**C**) until this sting punctures into skin, resulting in toxic fluids injection by pressure difference (**D**). See text for further explanation.

Once the stinging hair breaks off, the fluids with toxins should be exuded into the penetrating skin immediately within a very short time. The status of fluids in broken stinging cells and its eliciting force for penetrating skin can be explained by capillarity and pressure differences, respectively. In broken stinging cells, the status of fluids can be illustrated with a simplified assumption that the stinging cell is like a vertical water-filled tube placed in water (**Fig. 3A**), in which the water will be pulled up along the tube by capillary force. The height of water (h) in the tube could be formulated as follows:

$$h = \frac{2T\cos\theta}{\rho g r}$$

where T is surface tension (=72.0 mN/m at 25°C), θ is the contact angle between the water surface and the wall surface (**Fig. 3B**), ρ represents the density of liquid (=1000 kg/m^3), g is the gravity constant (=9.8 m/s^2), and r is the radius of the liquid tube. Assuming that the radius is 5 μm (10^{-6} m), similar to the tip radius of the sting (Fu *et al*. 2006), and that the contact angle is equal to 20° (0.35 radian), the height of the liquid could be elongated to 2(0.072) [cos(0.35)]/[(1000) (9.8)(5×10^{-6})] = 2.76 m, if such a long column exists. However, because the height of the sting (h' in **Fig. 3C**) is usually limited to a few mm, the liquid surface will be nearly flattened (θ~90°) at the broken tip (**Fig. 3C**). Upon penetration through the stratum corneum of the skin, the broken tip will connect to the intercellular space of the epidermis or dermis, in which the pressure (P' in **Fig. 3D**) is significant lower than atmospheric pressure (P) (**Fig. 3D**). This "pressure sink" may facilitate the toxic fluids spreading out in the intercellular space. This hypothesis may explain the observation that following a light touch with a needle, little drops of cell-sap exudes or even forcibly ejects immediately, as well as why the same hair can sting twice sequentially (Haberlandt 1914).

For sting hair regeneration, the cell membrane of the sting would be fused at the broken surface no matter how much the extent of upper stinging hair was fractured. Furthermore, in a mutilated stinging hair, a new and tender membrane develops upward to a point, without developing into a fully regular shape (**Fig. 1L**; Küster 1925). The reconstitution membrane (filling mass formation) was shown to contain calcium carbonate and cellulose (Korn 1944). However, whether the regenerated stinging hairs have stinging functions has not been reported.

RESPONSE TO STINGING REACTOIN: HUMAN'S VIEW

To date, there is only one detailed clinical study on skin responses upon being stung by stinging hairs (Oliver *et al*. 1991). According to Oliver's study, the wheal-and-flare reaction was maximized at about 5 min, accompanied by a stinging sensation, and later by pruritus. The urticaria responses faded in 1-2 h, but a persistent tingling paraesthesia could last up to 12 h. According to this description, the pathophysiological response can be grouped into two categories: one is urticaria, histological changes of skins, and the other, pain sensation related to nociceptive neurons. Although few studies specifically focused on the response of stinging hairs, molecular mechanisms of urticaria and nociception derived from other aspects can be taken into discussion. Because some toxins in the stinging hairs are also mediators of nociception and urticaria, understanding the basis of those mechanisms may provide a reference regarding the toxicity of the stinging hairs.

Nettle-induced urticaria

Urticaria is dermal edema in response to molecules released from mast cells (Hennino *et al*. 2006). Two kinds of urticaria can be distinguished by activators of mast cells: immunological and non-immunological urticaria. Immunological (allergic) urticaria is a hypersensitive reaction mediated by antibodies, mainly IgE, and/or T cells, while non-allergic urticaria results from other activators (Hennino *et al*. 2006). Nettle-induced urticaria could be regarded as the latter one, because the recently identified sting toxins are all small chemicals, not macromolecular allergens or antigens (with one exception that allergens were found in *Wigandia*; see Reynolds *et al*. 1989). However, a close association of mast cells with lymphocytes and dendritic cells in nettle-induced urticaria (Oliver *et al*. 1991) may imply that antibodies or T cells are involved. Interestingly, this cellular triad among mast cells, lymphocytes and dendritic cells was also found in the inflammatory response of drug-induced acute urticaria (Criado *et al*. 2006). The physiological significance of this cellular triad is still not understood.

There are three physiological consequences in response to mast cell activation: degranulation, and phospholipid metabolism, cytokine and *de novo* synthesis of chemokine (**Fig. 4**). Degranulation occurs at early phases and causes edema, and the latter two induce leukocyte infiltration at late phases. Histological studies demonstrated that mast cells were activated in nettle-induced urticaria. After skin contact with a stinging hair, degranulation of mast cells was observed at 5 min and 12 h (Oliver *et al*. 1991). Many mediators will be released from mast cells, but different inducing agents may have different signaling pathways for mast activation. It should be noticed that some toxins themselves identified in stinging hairs are also mediators of mast cells. Specifically, histamine and serotonin can be released from degranulation; leukotrienes and platelet-activating factors can be produced by lipid metabolism (**Fig. 4**) (Gilfillan and Tkaczyk 2006; Hennino *et al*. 2006). Although those mediators can be released from both mast cells and stinging cells, however, microdialysis studies showed no LTC_4 and weak histamine release in nettle-induced urticaria (Taskila *et al*. 2000). Furthermore, nettle hair extracts did not activate histamine-releasing from isolated mast cells (Oliver *et al*. 1991). The inducers and consequences of mast cell activation in nettle-induced urticaria need further clarification.

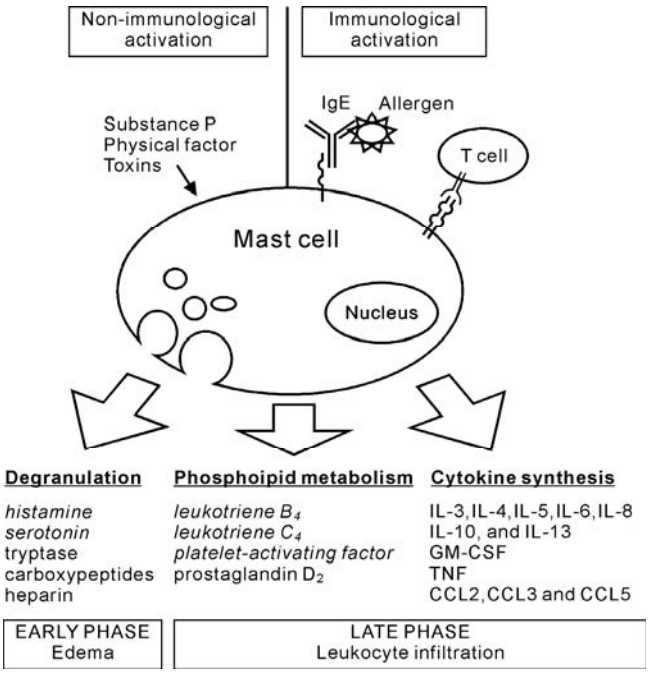

Fig. 4 Mast cell activation and responses. Mast cells can be activated by either immunological or non-immunological factors, resulting in three releasing mechanisms (underlines) in early or late phase. Below the corresponding releasing mechanisms are listed some mediators, in which the mediators *in italic* are chemicals also found as toxins in the stinging hairs. Adapted from Hennino *et al*. 2006.

Pain sensation

Pain is a complex sensation affected by noxious stimuli to nociceptors, cognitive and emotional processing by the brain. Pain messages are initiated by primary sensory fibers, including Aδ fibers and C fibers, which mediate for rapid, acute pain and delayed, diffuse pain, respectively (Julius and Basbaum 2001). There are many types of nociceptors for various types of pain. Aδ nociceptors have two classes, responding to intense mechanical nociceptors; C-fiber nociceptors are also polymodal, responding to noxious thermal, mechanical, and chemical stimuli (Julius and Basbaum 2001).

Of the sting toxins discussed above, most of them have been associated with pain signaling. Acetylcholine, histamine and serotonin may elicit pain by activating nociceptors directly, while serotonin and sustained proton (low pH) may alter neuronal excitability directly by interacting with ion channels on the nociceptor surface (Julius and Basbaum 2001; Schmelz *et al*. 2003). In inflammatory injuries, nociceptors may also elicit mediators like substance P to induce mast cell activation (Julius and Basbaum 2001). It should be noticed that the itch (pruritus) sensation may also be mediated by histamine or low pH via different receptors present in skin; namely, histamine induce itch via H1 receptor and low pH (pH = 3) induce burning pruritus via TRPV1 (Stander and Schmelz 2006). To date, no data with regards to molecular mechanism of nociceptors were available in nettle-induced urticaria. Further investigation on the mechanism of pain and itch sensation may explain the immediate stinging sensation and the persistent paraesthesia, respectively, of the skin after being stung.

PERSPECTIVES

Although stinging hairs have been studied for more than two centuries, many questions remain unresolved; the mechanism of stinging reactions seems complicated. Although a stinging hair seems simple to our understanding, its morphology and chemical toxins, when studied and discussed in depth, are more complicated than we think. Thurston and his colleague (1974, 1976, 1979) provide a nice studies about fine structure, and silica deposition of the stinging hairs and a thorough review (Thurston and Lersten 1969) after Haberlandt (1886).

Toxin identification of the stinging hairs requires close integration among stinging hair basis, analytical chemistry, and clinical expertise. Some comments in the following sections are suggested in further studies of sting toxins.

Pure extract source. Since the toxic effect is elicited by releasing sting fluids, toxins should be obtained from stinging cell extracts. Nevertheless, some previous studies used leaf tissues or whole plant extracts as materials (Saxena *et al*. 1965, 1966; Antonopoulou *et al*. 1996). In addition, liquid filtrates from forceps-removed stinging hairs in buffers (Emmelin and Feldberg 1947; Collier and Chesher 1956; Czarnetzki *et al*. 1990) may be mixed with the cytoplasm of other cells, such as pedestal cells and epidermal cells. Dipping fluids from tip-broken stings into buffers or water is probably one better method to obtain purer extracts (Fu *et al*. 2006).

High-sensitive analytical method. The most important criterion for sting toxin identification is whether the toxins of the collected stings are able to be detected. Since sting collection is fairly time-consuming, the counterbalance between numbers of stinging hairs and limits of analytical instruments is critical and requires preliminary trials. Moreover, data of weights or concentrations per sting are necessary to provide further toxic tests. Sting fluid volume needs to be determined for concentration calculations, although the vol-

ume of one stinging hair is around 4-9 nL in some species of *Urtica* (Emmelin and Feldberg 1947; Fu *et al*. 2006).

Resembled clinical test. Both conventional prick tests and intradermal injections cannot satisfy the resemblance with skins injected by stinging hairs, because a prick with chemicals does not match an injection, while the inner diameter of the smallest syringe needles (~100 μm at 33-gauge) is ten-fold of the thinnest diameter of the sting tip (~10 μm) in *U. thunbergiana* (Fu *et al*. 2003). New advanced microneedle arrays with a 10-20 μm injection diameter and precise volume control (Prausnitz 2004) may provide a good analogy to stinging hairs on the leaves, facilitating comparison between application of stinging hairs and delivery of identified toxins.

Although many anatomical observations of stinging hairs had been done, they need to be further investigated due to newly recognized toxins and more advanced technology. To understand the highly regulated systems in the stinging hair, some interesting topics deserve further investtigation. The architecture of the sting is an amazing design, but the molecular mechanisms of stinging hair ontogeny, namely, gene and protein regulation, are still unknown. The role of calcium in the stinging hair may also be an important topic. Oxalic acid in the stinging cells (Fu *et al*. 2006) without forming calcium oxalate deposits indicates that calcium and oxalic acids do not co-localize in the stinging cells. Besides, calcium deposits in the basal region of cell walls (Sowers and Thurston 1979) may limit the calcium circulating in the stinging hair; existence of calcium carbonate found in the sting (unpublished) provides a hypothesis that calcium may react with toxin synthesis byproducts, carbon dioxide, to form calcium deposits instead of calcium oxalate.

It is amazing that the identified sting toxins can not totally explain nettle-induced urticaria until now. Either some identified toxins cannot elicit the same reaction as that by stings, or the effective dosage to elicit skin responses was much higher than that by single or even multiple stinging hairs. It is possible, however, that the mixture of toxins in stings evokes more effective reactions than those summed by individual toxins. Besides, the sting hair injection might involve another physiological mechanism different from that of intradermal injections or skin pricks, resulting in more effective toxin responses. While much of the knowledge from stinging reactions is still fragmented and many aspects are still unresolved, the naturally-produced, microneedle-like structures – stinging hairs looking simple but acting complex, are still worthy of study.

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