Neuropathology of Skin Denervation in Acrylamide-Induced Neuropathy

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Previous studies have established the neurotoxicity and pathology of acrylamide to large-diameter nerves. It remains unclear (1) whether small-diameter sensory nerves are vulnerable to acrylamide and (2) if so, how the pathology evolves during intoxication. We investigated the influence of acrylamide on small-diameter sensory nerves by studying the pathology of sensory nerve terminals in the skin. The neurotoxic effects of acrylamide (400 ppm in drinking water) on mice were assessed by immunostaining the skin with protein gene product 9.5, a ubiquitin C-terminal hydrolase, particularly useful for demonstrating cutaneous nerve terminals. Within 5 days of acrylamide administration (the initial stage), epidermal nerves showed two major changes: (1) terminal swelling and (2) increased branching. There was a progressive reduction in epidermal nerve density (END) thereafter. Fifteen days after acrylamide intoxication (the late stage), reduction in END became evident (25.22 ± 2.19 fibers/mm vs 41.74 ± 2.60 fibers/mm in control mice, P < 0.003). At this stage, there was significant dermal nerve degeneration with ultrastructural demonstrations of vacuolar changes. These findings establish the pathological consequences of acrylamide neurotoxicity in cutaneous sensory nerves with far-reaching implications: (1) providing an animal system to study “dying-back” pathology of nociceptive nerves and (2) forming the ultrastructural foundation for interpreting the pathology of cutaneous nerve degeneration in skin biopsies.

INTRODUCTION

Sensory neurons are classified according to differences in their cytoskeletal organization, functions, and neurotrophin dependency (Snider, 1994; Chen et al., 1999). Peripheral axons of small-diameter sensory neurons terminate in the epidermis of the skin and are responsible for sensing noxious stimuli in the environment. Terminals of nociceptive nerves are difficult to visualize by conventional morphological techniques (Griffin et al., 2001). Neuropathies affecting nociceptive nerves are common in clinical settings; these include diabetic neuropathy (Kennedy & Wendelschafer-Crabb, 1996) and neuropathy associated with human immunodeficiency virus infection (McCarthy et al., 1995; Polydefkis et al., 2002a,b). By immunostaining the cutaneous tissues with various neuronal markers, several groups including ours have studied small-diameter nerves in the epidermis at the level of both light and electron microscopies (Kennedy and Wendelschafer-Crabb, 1993; Hsieh et al., 1996; Lin et al., 1997). These studies suggest that the early pathology of cutaneous nerve degeneration after mechanical injury begins from epidermal nerve terminals (Chiang et al., 1998; Hsieh et al., 2000). However, there has been a lack of experimental systems to study the distal pathology in small-fiber sensory neuropathy.

Several lines of evidence indicate that small-diameter nerves may be affected by acrylamide intoxication. For example, the response of sweat glands to pilo-
carnine, a cholinergic agonist, is reduced in acrylamide-intoxicated mice (Navarro et al., 1993). Unmyelinated axons in the vagus nerve and in the enteric nervous system degenerate after intoxication with acrylamide (Post, 1978; Belai & Burnstock, 1996). Acrylamide affects the most distal parts of central and peripheral axons at the beginning of intoxication as an experimental system of “dying-back” neuropathy (Schaumburg et al., 1974; Tsujihata et al., 1974; Miller & Spencer, 1985; Ko et al., 1999). Early neuropathological features include swelling of axonal terminals, while nerve degeneration is a late event in acrylamide neurotoxicity (Prineas, 1969; Suzuki & Pfaff, 1973; Ko et al., 1999). Potential mechanisms of acrylamide neurotoxicity include enhanced calcium entry (LoPachin & Spencer, 1985; Ko et al., 1999). The neuropathology of nociceptive nerve terminal degeneration in the skin, however, has never been evaluated in previous studies of acrylamide intoxication.

MATERIALS AND METHODS

Acrylamide Intoxication

Intoxication with acrylamide (Merck, Darmstadt, Germany) began with groups of 3-week-old male ICR mice following the established protocol (Bradley & Asbury, 1970). After acrylamide administration, there were characteristic and progressive changes in motor functions, morphology, and electrophysiology. The pathology of motor nerve terminals was described previously (Ko et al., 1999). This report specifically addresses the degeneration of cutaneous nerve terminals. Acrylamide was added to the drinking water (400 ppm) throughout the experimental period. Mice were housed in plastic cages (five per cage). In our preliminary study, the water intake by each mouse was 2.5 ± 0.3 ml/day, and the calculated dose of acrylamide ingested was 91.8 ± 20.6 mg/kg/day. Control mice were given tap water. Experiments were carried out in accordance with Use of Animals in Toxicology, and NIH guidelines (Guide for the Care and Use of Laboratory Animals, NIH Publication No. 86–23, 1985), the European Communities Council Directive of 24 November 1986 (86/609/EEC), and the guidelines established by the Animal Committee of National Taiwan University, Taiwan.

Acrylamide-intoxicated mice had characteristic progression of gait difficulties and limb weakness as defined before (Ko et al., 1999, 2000). Within 5 days of acrylamide intoxication, mice became ataxic, which was designated the initial stage. Hindlimb weakness became obvious 6–9 days after intoxication (the early stage). The weakness progressed, and forelimbs were affected in the late stage, beginning after 13 days of acrylamide intoxication. Each stage is associated with reduced retention time on the rota-rod test and characteristic pathology in neuromuscular junctions (Ko et al., 1999). Because neurological symptoms, physiological changes, and pathological signs are so distinct and correlated in each stage, temporal changes in skin innervation after acrylamide intoxication follow the same designation of motor deficits for each stage. We therefore sampled tissues on Day 5 (the initial stage), Day 10 (the early stage), and Day 21 (the late stage) of acrylamide intoxication (Table 1). At each time point, intoxicated mice showed characteristic gait and motor weakness fulfilling the definition as described.

Light Microscopic Immunocytochemistry

For immunocytochemistry of frozen microtome sections (Hsieh & Lin, 1999), mice were fixed with an intracardiac perfusion of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Footpads of the hindpaws were fixed for another 6 h, and 30-μm frozen sections were labeled sequentially. Sections were then processed for immunostaining. After quenching with 1% H2O2 in methanol, and blocking with 5% normal goat serum, sections were incubated with rabbit antisem to protein gene product 9.5 (PGP 9.5, UltraClone, UK, at a 1:1000 dilution in 1% normal goat serum) for 16–24 h. PGP 9.5, a ubiquitin carboxyhydrolase, is particularly useful for demonstrating unmyelinated nerves in the skin (Lin et al., 1997). After
rinsing in the phosphate buffer, sections were incubated with biotinylated goat anti-rabbit IgG for 1 h and in avidin-biotin complex (Vector, Burlingame, CA) for another hour. The reaction product was demonstrated by 3,3′-diaminobenzidine (Sigma, St. Louis, MO). Sections were then counterstained with toluidine blue to delineate the border between the epidermis and the dermis. To ensure adequate sampling, every fourth section of each tissue was chosen for PGP 9.5 immunostaining, and there were 5–6 immunostained sections for each tissue.

### Quantitation of Epidermal Innervation

Epidermal innervation was quantified according to modified protocols in a coded fashion (Chien et al., 2001). PGP 9.5-immunoreactive nerves in the epidermis of each footpad were counted at a magnification of 400× with a Zeiss Axiophot microscope (Carl Zeiss, Germany). The total length of the epidermis along the upper margin of the stratum corneum in each footpad was measured with Image-Pro PLUS (Media Cybernetics, Silver Spring, MD). Each individual nerve with branching points inside the epidermis was counted as one. The number of branching points for each epidermal nerve was also counted (Figs. 1A and B). The number of epidermal nerves with a given number of branching points per unit length of the epidermis was designated as the component epidermal nerve density (END) for that number of branching points (fibers/mm). An END histogram was constructed according to component epidermal nerve densities and branching points (Fig. 1C). The mean number of branching points of all epidermal nerves in that section was then calculated, for example, 0.31 points/fiber in Fig. 1C. The sum of all component epidermal nerve densities regardless of the number of branching points was designated the total END, as 44.09 fibers/mm in the example of Fig. 1C. Every fourth section for each tissue was quantified. Thus the value represents the mean of 5–6 sections for each mouse.

### Morphometry of Epidermal Nerve Varicosities

Epidermal nerves were photographed with a Zeiss Axiophot microscope under a magnification of 1000×. Slides were coded, and the examiners were blinded to the coding information. Prints were enlarged with a final magnification of 4200× and captured with a Kodak DCS 420 digital camera (Eastman Kodak Company, Rochester, NY). The outline of each individual epidermal nerve varicosity was discernible under this magnification. On average, there were 10–30 varicosities for each epidermal nerve. Every third epidermal varicosity of every fourth epidermal nerve was sampled for measurement. The size of individual varicosity was measured with Image-Pro PLUS. The mean of these values represents the size of the epidermal nerve varicosities for that epidermal nerve. There were 587 epidermal nerve varicosities from 109 epidermal nerves in five control mice and 608 epidermal nerve varicosities from 116 epidermal nerves in six acrylamide-intoxicated mice of the initial stage.

### Conventional Electron Microscopy Study

Mice were perfused with 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Hind paws of both control and acrylamide-intoxicated mice were dissected. Tissues stayed in the same fixative overnight. After thorough rinsing in the buffer, tissues were post fixed in 2% osmium tetroxide for 2 h, dehydrated through graded ethanol, and embedded in Epon (Hsieh et al., 2000). Semithin sections were stained with toluidine blue. Selected areas were thin-sectioned and observed under a Hitachi electron microscope.

### Electron Microscopic Immunocytochemistry

The procedures of electron microscopic immunocytochemistry were the same as those described previously (Lin et al., 1997). Briefly, paraformaldehyde-fixed tissues were sectioned on a vibratome. Free-
floating sections were incubated with an antibody against PGP 9.5. After the avidin-biotin complex procedure and the reaction with 3,3’-diaminobenzidine, sections were post fixed in 2% osmium tetraoxide for 2 h, dehydrated in ethanol, and embedded in resin. Selected areas were thin-sectioned, doubly stained with uranyl acetate and lead citrate, observed under a Hitachi electron microscope, and photographed.

Statistical Analysis

There were six mice at different stages after acrylamide intoxication in each study including light microscopic immunocytochemistry, conventional electron microscopy study, and electron microscopic immunocytochemistry. Three stage-matched control mice were used in each study. All quantitation was done in a blinded manner. Data are presented as the mean ± SEM and analyzed with SPSS for Windows (SPSS, Chicago, IL) and GraphPad Prism (GraphPad Software, San Diego, CA). For data passing the normality test, analyses included t test and ANOVA with post hoc test among control mice and acrylamide-intoxicated mice of different stages. If the data did not follow a Gaussian distribution or the data number was less than 3, nonparametric tests (Mann-Whitney U test for two groups and Kruskal-Wallis test with Dunn’s post test for multiple groups) were performed. In preliminary analysis, we found that epidermal nerve densities (component END and total END) and mean branching points were similar between the three groups of stage-matched control mice (P = 0.15–0.79). Data of these mice were therefore pooled together as the group of control mice in Table 2. Any difference with P < 0.05 was considered statistically significant.

RESULTS

Skin Innervation in Acrylamide Intoxication

In normal mouse skin, there was rich innervation of PGP 9.5 (+) nerves in the epidermis. Individual PGP 9.5 (+) nerves were grouped into fascicles in the dermis. PGP 9.5 (+) dermal nerves paralleled the epidermal-dermal border and formed the subepidermal nerve plexuses. Individual nerves then ascended into the epidermis perpendicular to the basement membrane. Typical epidermal nerves had a varicose appearance with some branching in the suprabasal layers, and they terminated in the granular layer of the epidermis (Fig. 2A).

Within 5 days of acrylamide intoxication (the initial stage), epidermal nerves and dermal nerves were still readily demonstrated with PGP 9.5. They had the same morphology as epidermal nerves and dermal nerves in control mice except that the branching patterns of the epidermal nerves had become more elaborate at this stage (Fig. 2B, Table 2). The number of epidermal nerves was significantly reduced 21 days after acrylamide intoxication, i.e., in the late stage (Fig. 2C and Table 2).
and therefore the data were pooled together.

branching points of the three groups did not statistically differ by Kruskal-Wallis test with Dunn’s post test for the three groups (P = 0.15-0.79), and therefore the data were pooled together.

The mean number of branching points was 41.74 ± 0.21 in acrylamide neurotoxicity, we quantiﬁed branching patterns and densities of epidermal nerves. The mean number of branching points for each epidermal nerve increased by 76% (0.37 ± 0.06, P < 0.05 compared to control mice) by ANOVA and posthoc test; **P < 0.003 compared to control mice). In control mice, only 20.33% of epidermal nerves had 1 branching points (Fig. 3). The percentage of epidermal nerves with ≥1 branching points was signiﬁcantly higher at the initial stage (31.21 ± 2.33%) than at other stages (P < 0.003, Fig. 3). At the initial stage, the total END remained the same (43.31 ± 5.16, P = 0.9, Table 2).

At the early and late stages, there was progressive and significant reduction in total END (33.62 ± 2.05 with P < 0.03 in the early stage, and 25.22 ± 2.19 with P < 0.003 in the late stage). The mean number of branching points in the early and late stages remained the same as that in the control mice (0.31 ± 0.03 and 0.30 ± 0.03, respectively, P > 0.05).

Quantitation of Epidermal Nerves in Acrylamide Neurotoxicity

To further clarify the pathology of epidermal nerve degeneration in acrylamide neurotoxicity, we quantiﬁed branching patterns and densities of epidermal nerves. The mean number of branching points was 0.21 ± 0.02 points/fiber, and the total END was 41.74 ± 2.60 fibers/mm in control mice (Table 1). In the initial stage, the mean number of branching points for each epidermal nerve increased by 76% (0.37 ± 0.03, P < 0.003 compared to control mice). In control mice, only 20.33% ± 1.70% of epidermal nerves had ≥1 branching points (Fig. 3). The percentage of epidermal nerves with ≥1 branching points was signiﬁcantly higher at the initial stage (31.21 ± 2.33%) than at other stages (P < 0.003, Fig. 3). At the initial stage, the total END remained the same (43.31 ± 5.16, P = 0.9, Table 2).

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Ultrastructural Studies of Nerve Degeneration in Acrylamide Intoxication

Epidermal nerves became swollen at the beginning of acrylamide intoxication. In control mice, epidermal nerves had a typical varicose appearance in the epidermis (Fig. 4A). At the level of electron microscopy, epidermal nerve terminal had normal-looking, electron-dense organelles. PGP 9.5 (+)-immunoreactive products appeared compact and were evenly distributed inside the axoplasm (Fig. 4B). In acrylamide-intoxicated mice of the initial stage, some epidermal nerve terminals became swollen at the light microscopic level (Fig. 4C). Ultrastructurally, PGP 9.5-immunoreactive granules in the swollen varicosities appeared coarse and were pushed to the periphery. The axoplasm was occupied by expanded organelles and electron-lucent spaces, corresponding to granulovacuolar degeneration (Fig. 4D).

To demonstrate epidermal nerve swelling, we measured and compared the sizes of individual epidermal nerve varicosities between control mice and acrylamide-intoxicated mice of the initial stage. In control mice, the size of individual epidermal nerve varicosities was 1.64 ± 0.03 μm³. The value was signiﬁcantly larger in acrylamide-intoxicated mice of the initial stage (2.26 ± 0.07 μm³, P < 0.0001, Fig. 5).

Dermal nerves exhibited a characteristic pattern of degeneration just as did epidermal nerves after acrylamide intoxication. In control mice, individual PGP 9.5 (+) dermal nerves had a linear pattern of similar axonal caliber at the level of light microscopy (Fig. 6A). In acrylamide-intoxicated mice of the late stage, the PGP 9.5 immunoreactivity of dermal nerves became fragmented and globular (Fig. 6B), reflecting Wallerian-like degeneration (Griffith et al., 1995; Chiang et al., 1998). In control mice, groups of unmyelinated nerves were enclosed by a single Schwann cell at the ultrastructural level (Fig. 6C). In contrast, dermal axons of acrylamide-intoxicated mice had become swollen with dissolution of organelles and the appearance of vacuoles (Fig. 6D). These results are

### Table 1

| Component END (fibers/mm) according to the number of branching points |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|
| Branching points | 0 | 1 | 2 | ≥3 |
| Initial stage (6) | 29.79 ± 2.11 | 11.23 ± 0.86* | 2.08 ± 0.24** | 0.20 ± 0.08 |
| Early stage (6) | 24.66 ± 1.05* | 7.64 ± 0.53 | 1.18 ± 0.16 | 0.14 ± 0.05 |
| Late stage (6) | 18.62 ± 1.43** | 5.75 ± 0.53 | 0.77 ± 0.19 | 0.08 ± 0.05 |
| Control mice (9) | 33.59 ± 2.13 | 7.33 ± 0.31 | 0.70 ± 0.23 | 0.12 ± 0.06 |
| Total END (fibers/mm) | 43.31 ± 5.16 | 2.13 ± 7.33 | 0.53 ± 0.77 | 0.86** ± 2.08 |
| Mean branching points (fibers/fiber) | 0.03 ± 0.06 | 0.03* ± 0.08 | 0.03** ± 0.05 | 0.03 ± 0.03** |

*END: Epidermal nerve density.

**Initial stage: 5 days after acrylamide intoxication. Early stage: 10 days after acrylamide intoxication. Late stage: 21 days after acrylamide intoxication. (n) number of mice examined. There were three control mice for each stage. The component ENDS, total ENDS, and mean branching points of the three groups did not statistically differ by Kruskal-Wallis test with Dunn’s post test for the three groups (P = 0.15–0.79), and therefore the data were pooled together.

* P < 0.05 compared to control mice by ANOVA and posthoc test; ** P < 0.003 compared to control mice by ANOVA and posthoc test.

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reminiscent of the ultrastructural characteristics of nerve swelling and vacuolar degeneration in the early phase of nerve degeneration (Hsieh et al., 2000).

DISCUSSION

The present report examines the quantitative and sequential pathology of skin denervation after acrylamide intoxication at the level of light and electron microscopy. These findings have two far-reaching implications. First, the study provides an ultrastructural basis for light microscopic interpretation and quantitation of nerve pathology in skin biopsies. Second, the involvement of small-diameter sensory nerves extends the spectrum of acrylamide neurotoxicity, and also established an experimental system to study chronic neuropathy affecting small-diameter sensory nerves. Taken together, this information expands our understanding of nociceptive nerve pathology and the extent of examining sensory neuropathy with new approaches.

Pathology of Epidermal Denervation in Acrylamide Intoxication

Characteristic changes in the initial stage of acrylamide toxicity increased epidermal nerve branching and swelling of epidermal nerve terminals. Later in acrylamide intoxication, there is a significant reduction in epidermal nerve densities, consistent with the ultrastructural observations of epidermal nerve degeneration (Hsieh et al., 2000). Of course, the possibility that acrylamide directly stimulates early branching and swelling formation of epidermal nerves could not be excluded. Nevertheless, these findings also provide pathological criteria for the interpretation of skin biopsies, which has become a new approach to diagnose.
small-fiber sensory neuropathy (Kennedy & Said, 1999; Kennedy & Wendelschafer-Crabb, 1999).

The conventional interpretation of epidermal nerve densities is mainly based on the loss of PGP 9.5 (+) epidermal nerves (McCarthy et al., 1995; Kennedy et al., 1996; Pan et al., 2001). The disappearance of PGP 9.5 (+) epidermal nerves is presumed to reflect Wallerian-like degeneration. Alternatively, the absence of PGP 9.5 immunoreactivity may indicate phenotypical changes in epidermal nerves. Most studies on skin biopsies are based on light microscopic observations, and the ultrastructural correlations of these changes have not been systematically addressed. The present study together with our previous report provides compelling evidence of epidermal nerve degeneration at the ultrastructural level (Hsieh et al., 2000).

In human skin, there are signs suggesting epidermal nerve and dermal nerve degeneration in the early

FIG. 4. Epidermal innervation in acrylamide intoxication. Skin sections from control mice (A, B) and from acrylamide-intoxicated mice of the initial stage (C, D) were immunostained with protein gene product 9.5 (PGP 9.5) and observed with light microscopy (A and C) and electron microscopy (B and D). (A) In control mice, epidermal nerves have a varicose appearance. (B) Ultrastructurally, individual varicosities of control mice have normal organelles. PGP 9.5 (+)-immunoreactive products appear compact and are evenly distributed inside the axoplasm. (C) In the initial stage of acrylamide intoxication, epidermal nerve terminals have become swollen. (D) The size of epidermal nerve varicosities of mice in the initial stage is significantly increased. PGP 9.5-immunoreactive granules in the swollen varicosity appear coarse and are pushed to the periphery. The axoplasm is occupied by expanded organelles and electron-lucent spaces, reflecting granulovacuolar degeneration. Bars = 10 μm (A and C), and = 1 μm (B and D).
phase of neuropathy. These include epidermal nerve swelling, increased branching points of epidermal nerves, and fragmentation of dermal nerve varicosities between the control and the acrylamide-intoxicated mice within 5 days of administration (the initial stage). The mean size of epidermal nerve varicosities was 1.64 ± 0.03 μm² (n = 587) in control mice. The size of epidermal nerve varicosities increased by 37.8% after acrylamide intoxication (2.26 ± 0.07 μm², P < 0.0001, n = 608 in intoxicated mice).

FIG. 5. Epidermal nerve swelling in acrylamide intoxication. The graph is a histogram comparing the sizes of epidermal nerve varicosities between the control and the acrylamide-intoxicated mice within 5 days of administration (the initial stage). The mean size of epidermal nerve varicosities was 1.64 ± 0.03 μm² (n = 587) in control mice. The size of epidermal nerve varicosities increased by 37.8% after acrylamide intoxication (2.26 ± 0.07 μm², P < 0.0001, n = 608 in intoxicated mice).

Vulnerability of Cutaneous Sensory Nerves to Acrylamide

The finding of epidermal nerve degeneration after acrylamide intoxication expands the spectrum of acrylamide neurotoxicity. In previous studies, functions of small myelinated and unmyelinated nerves were considered relatively preserved in acrylamide neurotoxicity (Edwards et al., 1991). Occasional reports have indicated that acrylamide produces autonomic neuropathy in visceral nerves of the ileum, including a reduction of catecholaminergic nerves and of the content of catecholamine in the ileum (Belai & Burnstock, 1996). Autonomic innervation of the mesenteric vessels, particularly the calcitonin gene-related peptide (+) nerves, was affected by acrylamide in histochemical and pharmacological studies (Ralevic et al., 1991). In a study comparing motor and sudomotor nerves, 90% of acrylamide-intoxicated mice failed the rota-rod test by 15 days following the administration of acrylamide. Sudomotor dysfunction also developed at 30 days after acrylamide intoxication (Navarro et al., 1993). These data suggest that both small-diameter nerves and large-diameter motor nerves are affected after acrylamide intoxication.

Recent studies applying immunocytochemistry of the skin have indicated that small-diameter nerves can also be affected by neuropathies previously considered to be the large-fiber-type neuropathy. For example, cisplatin mainly damages large myelinated sensory nerves (Chaudhry et al., 1994; Gao et al., 1995). Intriguingly, epidermal innervation was also reduced in cisplatin-induced neuropathy (Verdu et al., 1999). There was a significant loss of cutaneous nerves as assessed by skin biopsy in patients with Friedreich’s ataxia, a sensory neuropathy traditionally considered to be the large-fiber type (Nolano et al., 2001). These findings suggest that the extent of involvement of “large-fiber neuropathy” could be much broader than what is expected according to current examinations. Skin biopsy with quantitative evaluation of epidermal innervation provides a new approach to understanding the spectrum of neuropathy (Barohn, 1998; Kennedy & Said, 1999).

Cutaneous nerve degeneration results in anesthesia or hyperalgesia (Lin et al., 2001). In rodents, most behavioral examinations of skin innervation depend on the integrity of both motor and sensory pathways, for example, the responses to heat, cold, and von Frey tactile hair stimulations (Hao et al., 2000; Callizot et al., 2001). The functional consequences of epidermal nerve degeneration in acrylamide intoxication are difficult to evaluate by these methods due to profound motor weakness. Further studies employing sophisticated means may be useful for examining the physiology of degenerated epidermal nerves in acrylamide neurotoxicity, such as laser-evoked potential studies (Agostino et al., 2000; Woolf, 2000; Cruccu et al., 2001; Ji & Woolf, 2001).

What is the site of acrylamide sensory neuropathy, neuronal cell bodies or nerve terminals? We suggest that nerve terminal injury may underlie the neuropathology, a demonstration of “dying-back” pathology.
in nociceptive nerves. This is based on two observations. First, neuropathological findings indicate swelling of epidermal nerve terminals developed in the early phase while the complete loss of epidermal nerves occurred in the late phase of Wallerian degeneration (Hsieh et al., 2000). Second, the number and volume of small-diameter neurons in the dorsal root ganglion were not affected by acrylamide intoxication, compared to large-diameter neurons (Tandrup & Braendgaard, 1994).

The mechanisms responsible for acrylamide neurotoxicity of small-diameter sensory neurons remain elusive. Recovery from acrylamide intoxication was accelerated by 4-methylcarechol, which stimulates the synthesis of nerve growth factor (Saita et al., 1996). Nerve growth factor is required for the development and maintenance of small-diameter sensory neurons (Snider, 1994). Epidermal innervation was reduced in leprosy, and there was a gradation of differences between affected and unaffected sides. There was a correlated loss of nerve growth factor immunoreactivity in the basal keratinocytes of skin, the presumed target cells of nociceptive nerves (Facer et al., 1998; Facer et al., 2000). Taken together, this finding may partially

FIG. 6. Dermal nerves in acrylamide intoxication. Skin sections of control mice (A and C) and mice 21 days after acrylamide intoxication (the late stage, B and D) were observed either after immunostaining with protein gene product 9.5 (PGP 9.5) (A, B) or using regular electron microscopy (C, D). (A) In control mice, PGP 9.5 (+) dermal nerves have a discrete linear shape, and occasionally are grouped in bundles. (B) In acrylamide-intoxicated mice, dermal nerves have become fragmented with a globular appearance. (C) In the dermis of control mice, groups of unmyelinated nerves are enclosed by a single Schwann cell. The axoplasm and organelles are normal. (D) In acrylamide-intoxicated mice, the proportion of intact of axons is variable. Some axons have become swollen with the dissolution of organelles and the appearance of vacuoles. Bars = 10 μm (A, B), = 1 μm (C, D).
explain the fact that small-diameter neurons are also vulnerable to acrylamide intoxication. More studies are required to elucidate the potential mechanisms for the susceptibility of small-diameter sensory nerves to acrylamide. Nevertheless, the present study outlines the pathological progression of cutaneous nerve terminal degeneration in acrylamide intoxication, and acrylamide intoxication provides a new system to study nociceptive nerve degeneration and to test therapeutic alternatives for sensory neuropathy.

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