Peripheral Pain Fiber Function Is Relatively Insensitive to the Neurotoxic Actions of Acrylamide in the Rat

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Peripheral Pain Fiber Function Is Relatively Insensitive to the Neurotoxic Actions of Acrylamide in the Rat. EDWARDS, P. M., SPOREL-ÖZAKAT, R. E., AND GISPEN, W. H. (1991). *Toxicol. Appl. Pharmacol.* 111, 43-48. Acrylamide causes degeneration of sensory nerve axons in a range of experimental animals and in humans. Evidence of selective vulnerability of large diameter fibers has been derived largely from histological and electrophysiological measurements. We have examined the effect of repeated doses of acrylamide on the function of small diameter peripheral pain fibers in rats by measuring the intensity of neurogenic edema initiated by chemosensitive nerve endings and assessing the sensitivity of the hind paws to a noxious stimulus. These effects have been compared to the impaired sensorimotor performance (landing foot spread) resulting from acrylamide treatment and the change in functional integrity of the pain fibers following surgical lesioning of the sciatic nerves. Our results show that the selective vulnerability of large over small diameter sensory fibers previously reported in the literature is reflected in the differential impairment of proprioceptive and pain-related functions in the rat. © 1991 Academic Press, Inc.

Chronic exposure to acrylamide is known to result in peripheral neuropathy of the dying back type in both humans and experimental animals (Cavanagh, 1964; Spencer and Schaumburg, 1974a,b). Sensory nerves are particularly susceptible to the toxin (Fullerton and Barnes, 1966; Hopkins and Gilliatt, 1971; Schaumburg *et al.*, 1974; Sumner, 1980).

A number of histological and electrophysiological studies have indicated that, in general, susceptibility to the toxic effects of acrylamide is higher in large than in small diameter fibers (Schaumburg *et al.*, 1974; Hopkins, 1970). However, the extent of the selectivity of acrylamide for large diameter fibers has been questioned by several groups (Post and McLeod, 1973; Goldstein, 1985; Satchell and Hersch, 1988). In this study, we have com-

pared the effect of acrylamide on functions that depend on sensory nerves from different diameter groups. The abnormal gait and limb positioning, which is the earliest and most marked abnormality in intoxicated animals, is probably related to failure of proprioceptive function following terminal degeneration of the large diameter IA afferents (Sumner and Asbury, 1975; Lowndes *et al.*, 1978). The deficit in limb positioning can be quantified by the landing foot spread (LFS) method described by Edwards and Parker (1977). We have compared this deficit with the function of two distinct populations of small peripheral afferent nerve fibers, namely the small, thinly myelinated A δ fibers and the small unmyelinated C fibers responsible for pain perception. The C fibers, by the release of substance P from their peripheral nerve endings, are also responsible for the initiation of the neurogenic edema of the skin caused by mustard oil

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TABLE 1
ACRYLAMIDE DOSING REGIMES

Protocol	No. doses	Frequency	Total dose (mg/kg)	Days from last dose to final measurement
A	7	3/week	350	3
B	7	3/week	350	13
C	8	2/week	400	4
D	8	2/week	400	7

Note. Each dose was 50 mg/kg body wt. Control rats for each protocol received saline following the same dosing regime.

(Lembeck and Holzer, 1979; Jancsó *et al.*, 1980). The extent of the edema, which can be measured by the extravasation of dye into the skin is proportional to the number of functional type C sensory nerve endings. Measurement of neurogenic edema together with assessment of the sensitivity of the hind paws to noxious stimuli allows quantitation of the effect of acrylamide on the most distal parts of small peripheral sensory nerve fibers. The effect has been compared with the abnormalities in landing foot spread caused by acrylamide and the change in functional integrity of the pain fibers at 3 and 13 days following surgical lesioning of the sciatic nerves.

METHODS

Animals, dosing, and surgery. Female Wistar rats weighing 150–200 g at the start of the experiment were used. They were housed in plastic cages on deep sawdust litter with free access to food and water. The temperature in the room was maintained between 19 and 24°C. The rats were given repeated intraperitoneal injections of acrylamide (50 mg/ml in water; 50 mg/kg body wt) or saline (NaCl; 0.9% w/v in water; 1 ml/kg body wt). To minimize general toxic effects, exemplified by hyperexcitability, weight loss, and increased mortality under anesthesia, a low frequency of dosing was used. To achieve a cumulative dose of 350 mg/kg, seven doses were given at a rate of three injections per week. In order to achieve a higher cumulative dose (400 mg/kg) eight doses were spread over a longer period by giving two doses per week. These dosing regimes also avoid the problem of local irritation at the injection site. Similar dosing regimes have been used by others in studies of acrylamide neuropathy

(Suzuki and Pfaff, 1973; Edwards and Parker, 1977; Gipon *et al.*, 1977; Jennekens *et al.*, 1979; Sidenius and Jakobsen, 1983; Brismar *et al.*, 1987; Logan and McLean, 1988; Sporel-Özkat *et al.*, 1990). The different dosing regimes and experimental protocols are shown in Table 1.

Surgical procedures were carried out under general anesthesia using sodium pentothal (30 mg/kg) administered intraperitoneally. A unilateral crush lesion was placed on the sciatic nerve in the upper leg according to the method described by De Koning *et al.* (1986). This method causes distal degeneration of all the nerve fibers in the sciatic nerve. In order to prevent possible sprouting of saphenous nerve endings, the saphenous nerves were transected on both sides and 5-mm portions removed to prevent regeneration. On the contralateral side, the sciatic nerve was undamaged and this served as the control. Measurement of neurogenic edema was carried out 3 or 13 days after surgery.

Landing foot spread. The degree of sensorimotor impairment produced by acrylamide was quantified by measuring the landing foot spread (Edwards and Parker, 1977) three times a week from the day of the first acrylamide injection until the end of the experiment. In summary, the rats were dropped from a horizontal position at a height of 30 cm. The position of the fourth digit of each hindlimb upon landing was marked and the distance between the pair measured. This was repeated for a total of four times for each rat, and the mean calculated.

Pain sensitivity. The sensitivity of the hind foot sole to pain was measured three times a week from the day of the first acrylamide injection until the end of the experiment or on Days 3 and 13 after surgery, as appropriate. In short, a small electrical current (in the range 0.1 to 0.5 mA) was applied to the plantar surface of the hind feet through two stimulation poles (De Koning and Gispen, 1987). A normal rat retracts its paw instantaneously when the skin of the foot sole closes the electrical circuit at the lowest amperage. Following transection of nerves to the hind paw, no response is observed at the highest amperage (0.5 mA). Responses at intermediate strengths of current indicative of

impaired nerve function are observed during recovery from surgical damage to the nerve. Previous studies indicated that repeated testing does not alter the response in this test (Bijlsma *et al.*, 1981).

Neurogenic edema. The animals were anesthetized using sodium pentothal, 30 mg/kg, administered intraperitoneally. An intravenous injection of 1% aqueous Evans blue (freshly prepared) was administered (0.5 ml/100-g rat) into the tail vein prior to application of 8% mustard oil in mineral oil (freshly prepared) to the dorsal surfaces of the hind paws. This noxious chemical was allowed to act on the skin for 20 min to elicit an inflammatory response. The animals were then decapitated and blood samples were collected from the neck. Dorsal skin portions from both feet were collected and weighed. All procedures with mustard oil were performed in a fume hood.

The dye was extracted from the skin for 5 days with a 1% solution of suramin (Bayer) in methanol 3 ml/100 mg skin (Jancsó-Gábor *et al.*, 1967). The dye content in plasma samples diluted 1:200 with water and in the skin eluate was measured spectrophotometrically at 620 nm. The extravasation was calculated as microliters of plasma/100 mg skin.

Data analysis. Preliminary studies showed that the magnitude of neurogenic edema was not normally distributed. In the surgical lesion experiments, the sciatic nerve was only crushed on one side, so the contralateral uncrushed side served as the control and the Wilcoxon signed ranks test was used to compare edema on the crushed and uncrushed sides. In the acrylamide experiment, the rats were randomly paired prior to measurement to enable the effects of acrylamide on different parameters in different experiments to be combined in a single figure expressing each parameter as a percentage of control. Statistical analysis was always performed on the raw data and a Mann-Whitney *U* test was used. Landing foot spread data is normally distributed and analysis of variance has been used to analyze the time-course (Fig. 2). For the sake of consistency, in Fig. 3 the same statistical method (Mann-Whitney *U* test) has been used for the landing foot spread as was applied to the neurogenic oedema. *p*-Values of <0.05, tested two-sided, were considered as statistically significant.

RESULTS

Surgical lesions. Three days following crush lesion of the sciatic nerve, application of a small noxious stimulus to the plantar surface of the foot sole failed to elicit a normal withdrawal response at 0.5 mA, indicating total anesthesia of the region. The ability of C fibers to release substance P in response to local application of a chemical irritant was, however,

not significantly reduced (Wilcoxon signed rank test, crush side compared to control side, $p = 0.068$) as indicated by mustard-oil-induced neurogenic edema (Fig. 1). The reduction in neurogenic edema was marked 13 days after the surgery (Wilcoxon signed rank test, crush side compared to control side, $p = 0.043$), at which time the foot sole was still totally insensitive as measured by the absence of withdrawal from a 0.5-mA stimulus.

Acrylamide treatment. Animals treated with acrylamide showed signs of neuropathy which were first subjectively detectable after four to five doses following either dosing regime. The predominant abnormality was in the gait and posture. Clumsiness, hind-limb spreading, a waddling or a high-stepping gait, and tail stiffness were all observed in some of the animals. These abnormalities became progressively worse until 1 to 2 weeks after cessation of acrylamide. The gait then began to normalize. The degree of abnormality quantified by the landing foot spread showed a similar time course (shown in Fig. 2 for the seven-dose regime used in protocols A and B). The landing foot spread of acrylamide-treated animals was significantly different from controls from after

FIG. 1. Neurogenic edema was measured in the dorsal surface of the hind paws on the control (□) and crushed (■) sides 3 and 13 days after sciatic nerve crush. The histogram shows the median and interquartile ranges (Sprent, 1981) of the values for five rats at each time point. The differences between control and crushed sides were significant at 13 days (Wilcoxon signed rank test; $p = 0.043$) but not at 3 days ($p = 0.068$).

FIG. 2. The effect of acrylamide on landing foot spread was measured as described under Methods. Values given are the mean $\pm$ SEM of six rats. Animals received acrylamide (50 mg/kg, ip) (●) or saline (○) three times a week (shown by the arrows) for a total of seven doses (dosing regime used in protocols A and B). The acrylamide-treated group was significantly different from the control group (ANOVA with repeated measurements: $F(1,10) = 65.6$, $p < 0.001$). Supplemental t test indicated the acrylamide-treated rats were different from controls from Day -3 (i.e., after five doses of acrylamide) until Day 16 after the last of seven doses of acrylamide.

the fourth dose until 16 days after the last of seven doses of acrylamide.

At no time during the intoxication or the recovery period did the rats fail to respond normally to the lowest noxious electrical stimulus (0.1 mA). Three days after seven doses of acrylamide, neurogenic edema was normal, although the landing foot spread (LFS) values were highly abnormal (Fig. 3, A). After 13 days, the LFS was still significantly elevated but neurogenic edema remained normal (Fig. 3, B). Using a slightly higher total dose, a small decrease in the neurogenic edema was observed 4 days after the last dose, but this was in no way comparable with the marked and highly significant increase in the foot spread (Fig. 3, C) at this time. By 7 days after eight doses, the neurogenic oedema in acrylamide-treated animals was normal, although the LFS remained highly abnormal (Fig. 3, D).

DISCUSSION

Our results indicate that at times at which the function of the large diameter propriocep-

tive sensory fibers (measured by landing foot spread) is severely impaired, functions served by the small diameter A δ and C fibers remain totally unaffected or only marginally compromised. Thus the functional parameters are in keeping with histological and electrophysiological evidence for the selective sensitivity of large diameter sensory nerve fibers (Schaumburg *et al.*, 1974; Hopkins, 1970). Crushing the sciatic nerve in the upper leg results in immediate total anesthesia of the hind paw but loss of C-fiber terminal response to local stimulation is not maximal 3 days after the injury. Devor *et al.* (1979) reported that failure of conduction in C fibers distal to a sciatic crush does not occur until 3–4 days after the injury, corresponding to the Wallerian degeneration of the disconnected axon. In view of the delayed decline in neurogenic edema following sciatic nerve crush (Fig. 1) and the observation that proprioceptive function continues to deteriorate after cessation of acrylamide treatment, we chose the 2 weeks immediately succeeding acrylamide exposure as the period when any effects on pain function were most likely to become apparent. Measurement of

FIG. 3. Neurogenic oedema (▨), landing foot spread (▤), and pain sensitivity (■) were measured 3 (A) and 13 (B) days after the last of seven doses of acrylamide and 4 (C) and 7 (D) days after the last of eight doses of acrylamide. The value for each treated rat is calculated as a percentage of its paired control and the mean $\pm$ SEM of these percentages is shown. The number of pairs is shown in parentheses. All statistical analyses were performed on the raw data. The asterisks indicate a statistically significant difference (Mann-Whitney U test) from the controls at the level of $p < 0.05$ (*), $p < 0.01$ (*).

neurogenic edema at intervals between 3 and 13 days after the last dose of acrylamide, during which time proprioceptive abnormalities rose to a maximum and then began to normalize, should have allowed for any slight difference in the time-course of loss of C fiber terminal function and only a small, transient effect was found.

The decrease in neurogenic edema suggested 4 days after eight doses of acrylamide deserves further study. Decreased function of the autonomic SP-containing nerves innervating the bladder has been described in acrylamide neuropathy (Kudlacz *et al.*, 1989). This deficit was rapidly reversed (within 7 days of the last dose) and in our study, neurogenic edema was also completely normal within 7 days. Such a rapid recovery suggests reversible terminal dysfunction rather than axonal degeneration followed by regeneration. The selective vulnerability of long, large diameter fibers has been deduced largely from histological and electrophysiological evidence of axonal degeneration. Ultrastructural abnormalities resulting from acrylamide treatment do not show the same selective distribution as the axonal degeneration (Cavanagh, 1982; Jennekens *et al.*, 1979). Our results demonstrate that functional impairment shows the same relative sensitivity of large over small diameter fibers as is apparent in the selective axonal degeneration. However, the possibility of less severe or more subtle changes unrelated to axonal degeneration should not be forgotten.

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