Bull. Environm. Contam. Toxicol. 27, 888–893 (1981)

Selective Effect on Peripheral Nerves After Subchronic Administration of Acrylamide

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Acrylamide has been widely used as a prototype compound in studies of neurotoxicity because its characteristic effects, both experimental and clinical, have been well documented (SPENCER & SCHAUMBURG, 1974b). The earliest neuropathologic effect is damage to the nerve terminals of large diameter axons. The degeneration proceeds centrally (CAVANAGH 1979) as multifocal lesions to the nerve axons (JENNEKENS et al. 1979). Electrophysiologically, the only reported change is in conduction velocity which slows after chronic exposure (LESWING & RIBELIN 1969) and seems to be more pronounced for sensory nerve conduction than motor conduction (HOPKINS & GILLIATT 1971). The slowed conduction has been correlated with a selective degeneration of the faster conducting large diameter fibers from the nerve bundle (FULLERTON & BARNES 1966).

Behavioral deficits (tremor, ataxia, etc.) occur prior to the neuropathy and depend on the accumulated total dose of acrylamide, rather than on the daily dose or length of exposure. In cats the neurological signs appeared when the animals were given an average total dose of 100 mg/kg, whether it was given in divided daily doses for several days or several months (KUPERMAN 1958 cf. SPENCER & SCHAUMBURG 1974a,b). Behavioral changes such as those produced by acrylamide suggest that there are also changes in nerve excitability which should be detected using electrophysiologic techniques. However, a careful examination of changes in nerve excitability during this period of early acrylamide induced behavioral toxicity has not been made. The purpose of this study was to determine the dose schedule which was necessary for detecting acrylamide-induced changes in nerve excitability using electrophysiologic techniques.

METHODS

Male Sprague-Dawley rats (150-260 grams) in groups of four were given daily doses of acrylamide for 1, 2, 3, 4, 5 or 6 days such that the total cumulative dose was always 100 mg/kg. Twenty-four hours after the last dose, each animal was anesthetized with urethane (1.5 g/kg) and the hindlimb dissected to expose the sural and sciatic nerves unilaterally.

Each nerve in turn was placed on pairs of stimulating and recording electrodes for recording the compound action potential by methods previously described (ANDERSON et al. 1981). Briefly, supramaximal stimuli of 0.02 msec duration were presented at

0007-4861/81/0027-0888 $01.20

15 second intervals. The following characteristics of the nerve compound action potential were recorded: conduction velocity of the fastest fibers, calculated from the onset of depolarization; conduction velocity of the largest subset of fibers in the bundle, calculated from the time of peak depolarization; duration, calculated from onset of depolarization to return to baseline; rate of depolarization, calculated in mV/msec; peak amplitude, calculated in mV; and waveform relative area, calculated as the ratio of area prior to peak amplitude divided by the area following peak amplitude. These parameters were calculated from each of 100 consecutive responses and then averaged.

The refractory periods of the sural and sciatic nerve action potentials were tested using twin pulses at stimulus intervals varying from 1 to 6 msec. The relative refractory period is an exponential function and was analyzed as a ratio of the second action potential relative to the first for each pair of responses, using the method of Roberts and Trollope (1979). The interstimulus intervals required for the second potential to reach 50% and 75% of its unconditioned control were calculated for each nerve.

RESULTS

None of the electrophysiologic characteristics of the sciatic nerve were affected by acrylamide treatment under any of the dosing schedules. There were, however, significant changes in sural nerve excitability. By splitting the action potential waveform into two parts (before and after peak amplitude) and calculating the relative area, we could standardize the responses between animals. Table 1 compares the relative area ratios for the two nerves. In the sciatic nerves from the control animals, the first half of the potential, which reflects the responses of the fast fibers in the bundle, contributed less than half the total responses of the population. In the sural nerves from control animals, the first and second areas made up almost equal segments of the waveform.

TABLE 1
THE DISTRIBUTION OF FAST AND SLOW FIBERS IN THE SCIATIC AND SURAL NERVES AFTER ACRYLAMIDE ADMINISTRATION

Acrylamide (mg/kg)	Fast Fibers/Slow Fibers (Mean ± SEM)	
	Sciatic Nerve	Sural nerve
0	0.743 ± .08	0.991 ± .08
16	0.649 ± .06	0.751 ± .09*
20	0.693 ± .09	0.891 ± .05*
25	0.717 ± .08	0.852 ± .10
33	0.729 ± .03	0.755 ± .08*
50	0.871 ± .05	0.773 ± .06*
100	0.691 ± .06	0.74 ± .09*

*$p < 0.05$ compared with control

Acrylamide did not alter the distribution of the sciatic nerve. However, in the sural nerve, acrylamide proportionately decreased the contribution of higher conduction velocity components to the action potential. This decrease was significant for all acrylamide treated animals except the 25 mg/kg dose schedule, in which there was a greater variability among the animals.

TABLE 2
EFFECT OF ACRYLAMIDE ON RELATIVE REFRACTORY PERIOD OF SCIATIC AND SURAL NERVES

Acrylamide (mg/kg)	Sciatic Nerve	Sural Nerve	Sural/sciatic (%)
	50% Relative Refractory Period (msec)		
0	2.3 ± .36	2.12 ± .62	92
16	1.91 ± .32	2.18 ± .29	114*
20	1.95 ± .39	2.96 ± .41	152*
25	2.48 ± .28	3.17 ± .46	129*
33	3.1 ± .51	4.13 ± .63	133*
50	2.17 ± .20	3.41 ± .42	157*
100	1.83 ± .20	3.31 ± .90	181*
	75% Relative Refractory Period (msec)		
	Sciatic Nerve	Sural nerve	Sural/sciatic (%)
0	3.49 ± .64	3.92 ± .88	112
16	3.12 ± .80	3.93 ± .58	126**
20	3.26 ± .70	5.29 ± .89	162**
25	4.1 ± .49	5.99 ± .66	146**
33	4.98 ± .98	6.13 ± .63	123**
50	3.53 ± .35	5.8 ± .69	164**
100	2.97 ± .33	6.47 ± 1.26	218**

*$\alpha < 0.003$

**$\alpha < 0.002$

In the control animals, the relative refractory periods of both nerves were nearly equal. After acrylamide treatment there was a significant prolongation of the refractory period of the sural nerve but not of the sciatic nerve. Table 2 shows the selective increase in the interstimulus interval required for 50% and 75% recovery of the sural nerve action potential. There was a significant increase, compared to the sciatic nerve refractory period, after each dose schedule of acrylamide.

The changes in sural nerve responsiveness did not seem to be a function of dosing schedule. There was no significant difference in the magnitude of the acrylamide effect whether the 100 mg/kg dose was given as a single injection or in divided doses over 6 days.

TABLE 3
AVERAGE DAILY CHANGE IN BODY WEIGHT DURING CHRONIC ACRYLAMIDE ADMINISTRATION

ACRYLAMIDE (mg/kg)	BODY WEIGHT (grams/rat/day)
0	15.0 ± 1.1
16	7.0 ± 0.5
20	6.6 ± 0.9
25	3.4 ± 1.0
33	4.9 ± 1.4
50	-3.0 ± 0.5
100	-11.6 ± 4.4

However, the weight loss in these animals was clearly a function of the dosing schedule. Table 3 shows that the animals receiving acrylamide in one dose lost weight over the 24 hour period. Progressively smaller effects were noted in the other groups, and the animals receiving 33 mg/kg or less per day actually showed a daily weight gain. However, the gains were not as great as those seen in the saline treated control animals.

DISCUSSION

The results show that changes in nerve action potential characteristics can be detected after as little as 24 hours exposure to acrylamide. Furthermore, this dysfunction seems to be selective for the sural nerve since no changes in the sciatic nerve were observed after exposure up to 6 days. Since the magnitude of the effect was the same whether the 100 mg/kg dose of acrylamide was given over 1 or 6 days, the effect on sural nerve activity seems to be more a function of the total cumulative dose rather than length of exposure.

The selectivity of effect on the sural nerve, a sensory nerve, may not be simply due to a difference in its function. This nerve was recorded at a point more distal than the sciatic nerve. Since the nerve terminal seems to be more sensitive to acrylamide (CAVANAGH 1979), the more distal recording site would be the first segment of the nerve affected by acrylamide. The sciatic nerve, which was recorded at a more proximal site, may not have been as vulnerable.

It is important to note that the changes in the relative area of the sural nerve action potential and in the relative refractory period were observed long before conduction velocity changes (due to neuropathy) would be expected. The slowing of conduction after several months of exposure has a histological basis (FULLERTON & BARNES 1966). However the loss of the fast frequency components of the sural nerve action potential in the present experiments most likely is the result of biochemical changes such as altered potassium and sodium conductance, membrane effects, or altered glucose metabolism which do not necessarily depend on structural damage. These biochemical effects, however, could be and in many cases are the precursors to structural damage (DEWAR & MOFFETT 1979).

Several biochemical mechanisms of acrylamide neurotoxicity have been proposed. Acrylamide significantly decreases protein synthesis (SCHOTMANN et al. 1977), inhibits neuron specific enolase (HOWLAND et al. 1980) and inhibits phosphofructokinase (SABRI & SPENCER 1980). It is not surprising that these changes in metabolism would alter nerve excitability, since the membrane expends a great deal of energy to maintain its function. As this process progresses, the axon's inability to repair itself would affect structural as well as functional integrity and a neuropathy would appear.

Our results confirm those of other workers (KUPERMAN 1958; SPENCER & SCHAUMBURG 1974a,b) who have reported that the onset of acrylamide toxicity is a function of cumulative dose. The changes in the sural nerve activity were of the same magnitude whether the drug was given in one dose or over a six day period. The toxicity of the drug, irrespective of its pharmacokinetics, thus appears to be progressive and cumulative.

Our results further indicate that the neurotoxicity of the sural nerve was not a function of the weight lost by these animals. Although acrylamide has an immediate effect on body weight, this effect of the drug is apparently independent of the effect on nerve tissue. The weight loss (or reduction from normal) was proportional to the magnitude of the daily dose, whereas the nerve dysfunction was present and of the same magnitude whether the animals lost (100 mg/kg dose) or made small gains (16 mg/kg dose) in body weight. The sural nerve toxicity and weight loss are thus concurrent effects but probably unrelated.

These results show that electrophysiologic parameters can detect onset of neurotoxicity and can be used to distinguish between effects on subpopulations within the nerve bundle.

ACKNOWLEDGEMENTS

The author wishes to thank Mr. John Parker and Mr. Victor Church for assisting with the technical portions of this work. Supported by NIH grant ES01951.

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