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Action of acrylamide on selected enzymes of energy metabolism in denervated cat peripheral nerves

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The effect of acrylamide on selected glycolytic and citric acid cycle enzymes has been studied in denervated cat sciatic nerves in vitro and in vivo. The enzyme activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), neuron specific enolase (NSE), succinate dehydrogenase (SDH), and lactate dehydrogenase (LDH), has been examined in saline-perfused, desheathed and denervated peroneal (P) and tibial (T) nerves from cats treated with acrylamide (15 mg/kg/day, s.c.) or vehicle for 15 days. GAPDH activity in denervated P and T nerve stumps was 2.0- and 2.3-fold higher than normal P and T nerve values. GAPDH activity in Schwann cells in denervated P and T nerves of acrylamide-treated cats was markedly reduced (56% and 61% of untreated denervated nerves, respectively). LDH and SDH activities were unaffected by acrylamide and NSE activity was absent in denervated nerve stumps. Acrylamide (0.5 and 20 mM) inhibited GAPDH activity in denervated nerve homogenates by 67% and 29%, respectively. This study demonstrates that acrylamide inhibits GAPDH in Schwann cells. The significance of GAPDH inhibition by acrylamide in denervated nerves and its relation to distal axonopathy has been discussed.

The underlying mechanism(s) by which acrylamide produces distal axonal degeneration is unknown. Inhibition of certain neuronal glycolytic enzymes has been implicated in the pathogenesis of some toxic polyneuropathies[2–4,9,10,12,13]. Various reports have indicated that acrylamide neuropathy is associated with a reduction in the enzyme activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and neuron-specific enolase (NSE) in rat brain and peripheral nerve homogenates[2–4,9]. Since whole nerve and brain tissues were utilized in these studies, it was impossible to determine whether inhibition or loss of GAPDH activity occurred in the axonal or in the Schwann cell compartment. While direct methods to measure enzyme activity in these two compartments are unavailable, Schwann cells are separated from axons in distal stumps of transected nerves as a consequence of Wallerian degeneration[14]. Previous reports have demonstrated that Schwann cells comprise approximately 90% of the intrafascicular cell population of denervated nerve stumps, while axons are absent[6]. The present study employed this technique to examine in vitro and in vivo the effect of acrylamide on selected enzymes of glycolysis (i.e. GAPDH, NSE and LDH) and the citric acid cycle (SDH) in Schwann cells from denervated cat peripheral nerve. Preliminary results have been presented elsewhere[7,8].

Acrylamide (electrophoresis grade 99.9% purity) was purchased from Bio-Rad Laboratories, Richmond, VA. The following chemicals were obtained from Sigma Chemicals, St. Louis, MO: 2.6-dichlorphenol-indophenol, glyceraldehyde-3-phosphate, β-nicotinamide adenine dinucleotide (oxidized and reduced forms), DL-dithiothreitol, and potassium cyanide. All other chemicals were of analytical grade.

Cats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and the sciatic nerve exposed bilaterally at the level of the sciatic notch. The nerve was ligated with a pair of 4/0 sterile sutures, transected midway between the sutures and the cut ends sewn into adjacent muscle beds. Distal stumps of transected peroneal (P) and tibial (T) nerves were allowed to undergo Wallerian degeneration for a period of

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9 weeks. Animals were treated for 15 days with 15 mg/kg/day s.c. acrylamide in saline or an equivalent volume of saline.

Ten adult male and female mongrel cats, each weighing 2.5–3.5 kg, received 15 mg/kg/day acrylamide or saline s.c. for 15 consecutive days. Twenty-four hours after the last injection, anesthetized animals were perfused systemically with ice-cold saline to remove all blood cells, and the P and T nerves were excised bilaterally from the thigh to the ankle.

Desheathed intrafascicular tissue from normal or denervated (P and T) nerves was placed on an ice-cold, upturned Petri dish, minced with a razor blade, placed in 10 vols. of ice-cold 0.25 M sucrose and dispersed with a Brinkman polytron tissue homogenizer for 15 s (setting 10). The crude homogenate (CH) was centrifuged in a table-top centrifuge (800 *g* for 5 min at 4 °C) to remove nuclei, unbroken cells and other cell debris. The post-nuclear supernatant (S_1) was frozen at −73 °C for biochemical analysis.

Succinate dehydrogenase (SDH) (succinate: 2,6 dichlorophenol-indophenol oxidoreductase; EC 1.3.99.1) was determined spectrophotometrically with 2,6-dichlorophenol-indophenol as an electron acceptor by modification of the method of Veeger et al.[16]. Lactate dehydrogenase (LDH) (L-lactate: NAD oxidoreductase; EC 1.1.1.27) was assayed according to the method of Stolzenbach[15]. Enolase (2-phospho-D-glycerate hydrolase; EC 4.2.1.11) and NSE were assayed according to the method of Marangos et al.[5]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (D-glyceraldehyde-3-phosphate: NAD oxidoreductase (phosphorylating); EC 1.2.1.12) was determined with minor modification of the method of Sabri and Ochs[11].

Acrylamide inhibited GAPDH activity, in vitro, in the S_1 from denervated nerve tissue (pooled P and T) in a concentration-dependent manner (Fig. 1). GAPDH activity in denervated nerves was significantly reduced to 63% and 79% of control values with 2.5 to 20.0 mM acrylamide, respectively.

GAPDH activity in P and T nerve stumps was 2.0- and 2.3-fold higher than that of values from normal P and T nerve (Fig. 2). GAPDH activity was significantly reduced by 44% and 39%, respectively, in P and T nerve stumps from acrylamide-treated animals (Fig. 2).

NSE, LDH and SDH activities in the S_1 prepared from intrafascicular tissue of denervated nerves, from animals with and without acrylamide treatment, are listed in Table I. No alteration in LDH or SDH activities was observed in axon-free nerves from acrylamide-treated cats. NSE activity was absent in denervated nerves.

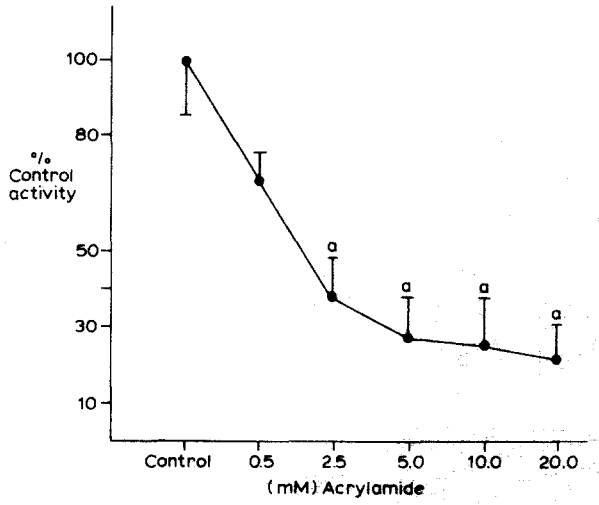

Fig. 1. Effect of acrylamide in vitro on GAPDH in denervated cat sciatic nerve. The S_1 from desheathed denervated nerve was preincubated with acrylamide (0.5–20.0 mM) for 20 min at 37 °C in a shaking water bath before determination of GAPDH activity. Each value is the mean ± S.E. % of 3–4 determinations run in triplicate. [a] $P < 0.05$.

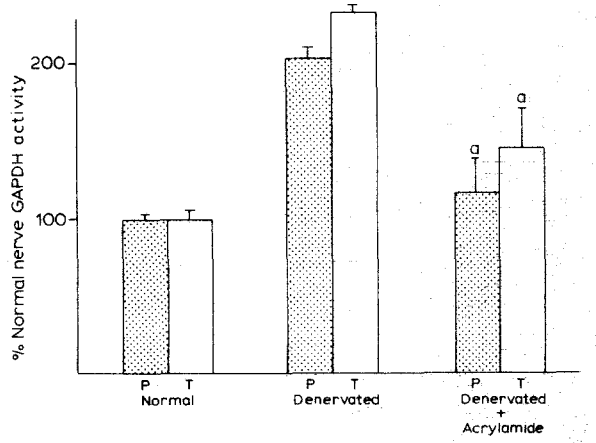

Fig. 2. Effect of denervation and acrylamide treatment on GAPDH activity in denervated P and T nerves. Each value represents the mean (expressed as % of normal-nerve GAPDH activity) ± S.E. % of 4 determinations run in triplicate. P, peroneal; T, tibial; a = significantly less than denervated; $P < 0.05$. GAPDH enzyme activity was expressed as micromoles NADH produced/min/mg protein. Actual values in control, denervated and acrylamide-treated peroneal and tibial nerves were as follows: normal P, 0.104; T, 0.095; denervated P, 0.213; T, 0.221; denervated (acrylamide) P, 0.120; T, 0.135.

TABLE I

Neuron-specific enolase, lactate and succinate dehydrogenase activities in denervated nerve treated with and without acrylamide

Animals were treated and tissue prepared as described previously. a = nmol succinate oxidized/min/mg protein; b = μmol pyruvate reduced/min/mg protein; c = 15 mg/kg, s.c. for 15 consecutive days. Each value represents the mean of 3–5 determinations run in duplicate. Parentheses = ± S.D. N.D. = not detected.

	SDH[a]	*LDH*[b]	*NSE*
Denervated	0.27 (0.06)	0.14 (0.01)	N.D.
Denervated + acrylamide[c]	0.24 (0.08)	0.16 (0.03)	N.D.

The goal of this study was to expand our understanding of the way acrylamide affects peripheral nerve function. Attention has been focused on the effect of this neurotoxin on selected enzymes of energy metabolism in the Schwann cell from denervated cat peripheral nerve, in vivo and in vitro.

The data show that Schwann-cell GAPDH activity increased over 2-fold in P and T denervated distal stumps over that of normal, untreated P and T nerves. Presumably, enhanced enzyme activity is due to the numerical increase in Schwann cells accompanying Wallerian degeneration. Schwann-cell GAPDH in normal nerves and axon-free distal stumps is susceptible to acrylamide to similar degrees both in vitro and in vivo. This suggests that the majority of GAPDH is located in the Schwann cell compartment rather than in axons. The sensitivity of GAPDH to acrylamide in normal nerve in vivo and in vitro has been reported elsewhere[2,3,9]. SDH and LDH activities were unaffected by acrylamide in denervated nerve, in vivo.

These studies indicate that: (1) acrylamide selectively inhibits enzymes of energy metabolism (GAPDH not LDH or SDH) in the Schwann cell; (2) NSE activity is absent in Schwann cells and therefore present in axons; (3) GAPDH activity is high in Schwann cells. Previous studies examining enzymes of energy metabolism in peripheral nerves have assumed that activity predominantly resides in axons. It is now apparent that the contribution of GAPDH activity in Schwann cells obscures changes that may occur in axons. The role of GAPDH inhibition in the generation of axonal neuropathy induced by acrylamide is therefore unknown.

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