Alterations in the Neutral Proteinase Activities of Central and Peripheral Nervous Systems of Acrylamide-, Carbon Disulfide-, or 2,5-Hexanedione-Treated Rats

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ABSTRACT

Proteinases are widespread in neuronal or nonneuronal eukaryotic cells. They are suggested to play an important role in the turnover of proteins in neuronal perikaryon and axon, and digestion of the transported cytoskeletal proteins in synaptic terminals. We examined the effect of acrylamide (50 mg/kg, ip), carbon disulfide (700 ppm, 9 h, 7 d a week), and 2,5-hexanedione (2,5-HD) (1% in drinking water) treatment of rats on mCANP (2 mM Ca²⁺), μCANP (0.1 mM Ca²⁺), and CINP (Ca²⁺-independent) activity in telencephalon + diencephalon (FB), rhombencephalon + mesencephalon (LB), spinal cord (SC), and sciatic nerve (SN). The proteinase activity was determined in the 30,000g supernatant fraction of tissues using ¹⁴C-methylated casein as the substrate. mCANP activity in FB, LB, and SC was inhibited only by acrylamide. Acrylamide or 2,5-HD treatment had no effect on μCANP and CINP activities of SN, whereas carbon disulfide enhanced μCANP after 15 d and CINP activity after 10 d. It is suggested that alteration in in vitro calpain activity shown by these chemicals may not be directly related to their neurotoxic effect. However, calpain may still be playing a role in this polyneuropathy by alteration in

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activity through inflow of Ca\(^{2+}\), release of Ca\(^{2+}\) from intracellular organelles, or other factors. Modification of cytoskeletal proteins making them more susceptible to proteases and the role of some other proteinase is also possible.

**Index Entries:** Acrylamide; carbon disulfide; 2,5-hexanedione; CANP; brain; rat; spinal cord; sciatic nerve.

**Abbreviations:** EDTA, ethylenediaminetetraacetic acid; EGTA, ethyleneglycol-bis(\(\beta\)-aminoethyl ether) \(N,N,N',N'\)-tetraacetic acid; 2,5-HD, 2,5-hexanedione; MAPs, microtubule-associated proteins; CINP, proteinase activity in excess of EGTA; \(\mu\)CANP, proteinase activity at 0.1 mM Ca\(^{2+}\); mCANP, proteinase activity at 2 mM Ca\(^{2+}\); FB, telencephalon + diencephalon; LB, rhombencephalon + mesencephalon; SC, spinal cord; SN, sciatic nerve; TCA, trichloroacetic acid.

**INTRODUCTION**

Acrylamide, carbon disulfide, \(n\)-hexane, and methyl \(n\)-butyl ketone are important industrial chemicals, and are known to produce central/peripheral neuropathy in human and other sensitive species. The neurotoxic effect of hexane and methyl \(n\)-butyl ketone is ascribed to their neurotoxic metabolite, 2,5-hexanedione (2,5-HD). The polyneuropathy produced by chronic or subchronic exposure to these chemicals is mainly characterized by weakness of the hindlimbs, followed by mild or severe ataxia (Spencer and Schaumburg, 1974, 1976; Pappolla et al., 1987; Abou-Donia et al., 1993). Histopathologically, this disease is accompanied by axonal swellings in preterminal nodes of Ranvier and Wallerian-type degeneration down these swellings (Prineas, 1969; Sayre et al., 1985). There are, however, some clinical as well as histopathological differences in the polyneuropathy produced by these chemicals (Gottfried et al., 1985; Sterman and Sposito, 1985; Shell et al., 1992).

Proteinases are suggested to play a role in the maintenance of axonal flow of fast- and slow-moving cytoskeletal proteins. They break down these proteins, including neurofilaments (NFs), on reaching the nerve terminals (Zimmerman and Schlaepfer, 1984). The injection of leupeptin into the optic tectum of goldfish resulted in the accumulation of NFs in synaptic terminals (Roots, 1983). The subsequent fate of the proteolytic fragments is not known, but it is suggested that they may constitute signals between the distal end of nerve and the perikaryon (Schlaepfer, 1987; Edwards et al., 1991).

Calpain now forms a family of proteinases consisting of at least six distinct members (Saido et al., 1994), although two forms of calcium-dependent neutral proteinase (CANP) activity have been found in virtually all eukaryotic cells. One form is activated at micromolar Ca\(^{2+}\) concen-