50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	PB89-129860

4. Title and Subtitle: Molecular Mechanisms of Diketone Neurotoxicity

5. Report Date: 88/02/08

6.

7. Author(s): DeCaprio, A. P.

8. Performing Organization Rept. No.

9. Performing Organization Name and Address: Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York

10. Project/Task/Work Unit No.

11. Contract (C) or Grant(G) No.
(C)
(G) R01-OH-01972

12. Sponsoring Organization Name and Address

13. Type of Report & Period Covered

14.

15. Supplementary Notes

16. Abstract (Limit: 200 words) Formation of the pyrrole adduct following either acute or chronic exposure of rats to 2,5-hexanedione (110134) (HD) was investigated. Physicochemical characterization of serum albumin and axonal cytoskeletal protein from HD treated animals was undertaken, and specific sites of lysine modification were elucidated. In-vivo protein binding of nonneurotoxic HD was characterized and the ability of 2,4-HD to influence the time course of 2,5-HD neuropathy by competitive binding was assessed. An examination was also made of in-vitro covalent protein amine binding of other neurofilamentous neurotoxins. Findings supported the hypothesis that pyrrole formation in neurofilament protein is a required step in gamma-diketone neuropathy. The rapid reorganization of the axonal cytoskeleton seen with direct application of 2,5-HD to nerve fibers may involve pyrrole formation but not crosslinking, that at high concentrations 2,5-HD appears able to react with previously formed pyrroles to form higher adducts, that the rate determining step of the reaction is attack of the enamine nitrogen upon the second carbonyl function to form the pyrrole, that in-vivo alterations may result in disruption of axonal cytoskeletal function, and that the related neurotoxins are also capable of covalently modifying lysine amino groups in protein while acrylamide may preferentially react with sulfhydryl moieties.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms: NIOSH-Publication, NIOSH-Author, NIOSH-Grant, Grant-Number-R01-OH-01972, End-Date-10-31-1987, Neurotoxic-effects, Neuropathology, Nervous-system-disorders, Metabolic-study, Protein-chemistry

c. COSATI Field/Group

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
		6
	22. Security Class (This Page)	22. Price
		A02

R1740

Final Performance Report

Research Grant 5 R01 OH-01972
Anthony P. DeCaprio, Ph.D.
February 8, 1988

Summary Statement:

The following specific aims were addressed in the funding period of this grant (5/1/84 - 10/31/87):

1. Detection and quantitation of pyrrole adduct formation in neural and nonneural proteins from rats receiving acute or prolonged exposure to 2,5-hexanedione (2,5-HD), and following cessation of exposure in order to assess clearance of the adduct.

2. Physicochemical characterization of serum albumin and axonal cytoskeletal protein from 2,5-HD-treated animals; elucidation of specific sites of lysine modification within these proteins.

3. Characterization of in vivo protein binding of non-neurotoxic 2,4-HD.

4. Assessment of the ability of 2,4-HD to influence the time-course of 2,5-HD neuropathy by competitive binding with protein amino groups.

5. Examination of in vitro covalent protein amine binding of other neurofilamentous neurotoxins, including carbon disulfide, β,β'-iminodipropionitrile (IDPN), and acrylamide.

As suggested in the original summary review statement for this grant, research efforts were concentrated on fulfillment of specific aims 1 and 2. Major progress was made towards accomplishment of these aims, and our most important findings are listed below. Progress was also made for specific aim 5. In general, our findings provide strong support for our hypothesis that pyrrole formation in neurofilament (NF) protein in a required step in γ-diketone neuropathy. The present work has provided much additional progress towards the elucidation of molecular mechanisms of action of the γ-diketones and other occupational neurotoxins.

List of Significant Results:

- Methodology for sensitive detection and quantitation of pyrrole adduct formation in tissue protein was perfected.

- Evidence for dose- and time-dependent pyrrolylation of axonal cytoskeletal proteins was obtained following exposure of rats to 2,5-HD.

- A decrease in the pyrrole concentration of such preparations was detected following cessation of exposure, consistent with the presence of clearance mechanisms within the axon capable of removing pyrrolylated protein to some extent.

- Pyrrole adduct formation in NF protein was detected and estimated to be in the 1-3 adducts/molecule range, suggesting the conversion of specific, highly vulnerable lysine groups.

- The formation of crosslinked derivatives of NF proteins with 2,5-HD exposure *in vivo* was revealed. The crosslinking appeared to be restricted to the NF-M and NF-H subunit proteins; those species involved in maintenance of normal NF-NF interaction within the nerve fiber.

- The protein crosslinking phenomenon in 2,5-HD neuropathy was demonstrated to be a free radical-mediated reaction probably involving radical addition to the pyrrole nucleus. The reaction was inhibited under a nitrogen atmosphere and in the presence of antioxidants, and was accelerated by radical initiators.

- Crosslinking was shown *in vitro* to proceed solely by pyrrole-pyrrole dimerization, thus excluding proposed mechanisms involving other amino acid sidechains.

- *In vitro* studies demonstrated a lag period between initial pyrrole formation and subsequent autoxidative crosslinking. This suggests that the rapid reorganization of the axonal cytoskeleton seen with direct application of 2,5-HD to nerve fibers may involve pyrrole formation but not crosslinking.

- *In vitro* studies revealed that lysine is the only amino acid in protein exhibiting significant reaction with 2,5-HD. In addition, at high concentrations, 2,5-HD appears able to react with previously formed pyrroles to form higher adducts.

- The mechanism of the pyrrolylation reaction involves the formation of an imine intermediate. The rate-determining step of the reaction is attack of enamine nitrogen upon the second carbonyl function to form the pyrrole. This confirms previously proposed mechanisms for the reaction.

- Pyrrolylation of protein results in conformational changes associated with translocation of the hydrophobic adduct to a less accessible location within the molecule. Thus, such alterations occurring *in vivo* may lead to disruption of axonal cytoskeletal function.

- The deuterated 2,5-HD derivative ($[D^{10}]$-2,5-HD) forms pyrroles at a slower rate *in vitro* and *in vivo*, and is less neurotoxic in the rat than native 2,5-HD. This is strong evidence for an absolute requirement for the pyrrolylation phenomenon in γ-diketone neuropathy.

- In vitro studies indicate that the related neurotoxins carbon disulfide and IDPN are also capable of covalently modifying lysine amino groups in protein, while acrylamide may preferentially react with sulfhydryl moieties.

Publications Derived from Research Grant OH-01972:

Research Papers:

DeCaprio, A.P. and O'Neill, E.A. (1985). Alterations in rat axonal cytoskeletal proteins induced by in vitro and in vivo 2,5-hexanedione exposure. Toxicol. Appl. Pharmacol. 78, 235-247.

DeCaprio, A.P. (1985). Molecular mechanisms of diketone neurotoxicity. Chem.-Biol. Interact. 54, 257-270.

DeCaprio, A.P. (1986). Mechanisms of in vitro pyrrole adduct autoxidation in 2,5-hexanedione-treated protein. Mol. Pharmacol. 30, 452-458.

DeCaprio, A.P. (1987). n-Hexane neurotoxicity: A mechanism involving pyrrole adduct formation in axonal cytoskeletal protein. Neurotoxicology 8, 199-210.

DeCaprio, A.P., Jackowski, S.J., and Regan, K.A. (1987). Mechanism of formation and quantitation of imines, pyrroles, and stable nonpyrrole adducts in 2,5-hexanedione-treated protein. Mol. Pharmacol. 32, 542-548.

DeCaprio, A.P., Briggs, R.G., Jackowski, S.J., and Kim, J.C.S. (1988). Comparative neurotoxicity and pyrrole-forming potential of 2,5-hexanedione and perdeuterio-2,5-hexanedione in the rat. Toxicol. Appl. Pharmacol., in press.

Invited Symposia:

DeCaprio, A.P. (1984). Molecular mechanisms of n-hexane neurotoxicity. In: Proceedings of the 14th Conference on Environmental Toxicology, Nov. 15-17, 1983. Air Force Aerospace Medical Research Laboratory, Dayton, OH; AFAMRL Publication TR-83-099, pp. 40-59.

DeCaprio, A.P. (1985). Hexane neuropathy: Studies in experimental animals and man. British Toxicology Society Autumn Meeting, University of Kent, Canterbury, U.K.; Sept. 25-27, 1985.

DeCaprio, A.P. (1986). n-Hexane neurotoxicity: A mechanism involving pyrrole adduct formation in axonal cytoskeletal protein. Mid-Atlantic Regional Meeting, American Chemical Society, Baltimore, MD; Sept. 2-5, 1986.

Book Chapter:

DeCaprio, A.P. (1987). Hexane neuropathy: Studies in experimental animals and man. In: Selectivity and Molecular Mechanisms of Toxicity. (F. DeMatteis and E.A. Lock, eds.), pp. 249-263; MacMillan Press, London.

Abstracts:

DeCaprio, A.P. and O'Neill, E.A. (1985). Alterations in rat axonal cytoskeletal proteins induced by 2,5-hexanedione exposure. Toxicologist 5, 199.

DeCaprio, A.P. (1986). Hexane neuropathy: Studies in experimental animals and man. Human. Toxicol. 5, 112.

DeCaprio, A.P. (1986). Mechanism of pyrrole autoxidation in 2,5-hexanedione-treated protein. Toxicologist 6, 189.

DeCaprio, A.P. and Jackowski, S.J. (1987). Comparative neurotoxicity and pyrrole-forming potential of 2,5-hexanedione and perdeuterio-2,5-hexanedione in the rat. Toxicologist 7, 130.