List of Abbreviations

CaM: Calmodulin

HRP: Horseradish peroxidase

MAP: Microtubule associated protein

MT: Microtubule(s)

Progress

1. The specific aim of determining whether MT disassembly and inhibition of assembly caused by Cd^{2+}, Hg^{2+}, and Pb^{2+}, in cultured 3T3 cells is due to binding to tubulin sulfhydryl groups or binding to and activation of CaM, was not achieved.

2. Methylmercury (CH_3Hg^+), instead of As^{3+}, was used as a model for adverse effects to MT due to tubulin sulfhydryl binding in the extracted cytoskeleton model system, and during the polymerization of purified MT proteins in vitro.

3. The results showing that, like Ca^{2+}, the inhibitory effect of Cd^{2+} on the polymerization of purified MT proteins containing tubulin and microtubule-associated proteins (MAPs) is enhanced by exogenously added CaM suggest that MAPs are involved in mediating the sensitivity of MT to Cd^{2+}-activated CaM.

4. The findings that Cd^{2+}, like Ca^{2+}, can support the binding of CaM to tubulin and MAPs in Western blots of purified MT proteins support the proposal that MAPs are important in the increased sensitivity of MT to Cd^{2+}-activated CaM.

5. The stimulation by Cd^{2+} of Ca^{2+}/CaM-dependent protein kinase II, resulting in MAP phosphorylation and MT disassembly and inhibition of assembly, was not investigated.

Significant Results

1. Cadmium chloride at concentrations between 10^{-5}M and 10^{-4}M causes microtubule disassembly and inhibition of MT reassembly, as visualized by indirect immunofluorescence, in cultures of quiescent Swiss 3T3 cells.

2. The MT networks of in situ detergent extracted cytoskeletons of cultured cells provide a model system for studying mechanisms of MT disassembly. The value of this model system is that the cytoplasmic MT network is assembled by living cells, but experiments can be carries out under "in vitro" conditions on in situ detergent extracted cytoskeletons.

3. Micromolar concentrations (10^{-5}M) of Ca^{2+} or Cd^{2+} cause MT disassembly in detergent extracted cytoskeletons of 3T3 cells. Like Ca^{2+}, the Cd^{2+}-

induced disassembly of detergent extracted MT is prevented by the CaM antagonists trifluoperazine and compound 48/80. The action of these CaM inhibitors depends upon the existence of CaM in its activated conformation following occupancy of the Ca^{2+}-binding domains by an appropriate cation. Thus, the prevention of Ca^{2+}, as well as Cd^{2+}-induced MT disassembly by CaM antagonists strongly suggests that MT disassembly caused by Cd^{2+} in detergent extracted cytoskeletons results from the binding and activation of CaM by Cd^{2+}.

4. Hg^{2+} and CH_3Hg^+ are more potent (10^{-6}M) than Cd^{2+} in causing MT disassembly in the extracted cytoskeleton. CaM inhibitors do not prevent CH_3Hg^+-induced MT disassembly, indicating that the CaM antagonists themselves have no MT stabilizing properties. In contrast, inconsistent results with CaM inhibitors and Hg^{2+} were obtained.

5. The <u>in vitro</u> polymerization of bovine brain MT protein (at 27°C) containing tubulin and MAPs is inhibited by 10^{-5}M-10^{-4}M $CaCl_2$ or $CdCl_2$, as indicated by extended lag times, decreased initial rates, and reduced final extents of MT polymerization. The inhibitory effect of Cd^{2+} on MT polymerization, like Ca^{2+}, is enhanced by exogenously added CaM. CaM in the absence of Ca^{2+} or Cd^{2+} has no effect on MT polymerization. In addition, this enhancement by CaM of Ca^{2+} or Cd^{2+}-induced inhibition of MT polymerization is reversed, in a dose dependent fashion, by compound 48/80. These findings strongly suggest that enhanced inhibition of MT polymerization by Cd^{2+} in the presence of CaM is due to the binding and activation of CaM by Cd^{2+}. In contrast, CaM had no effect on CH_3Hg^+-induced inhibition of MT polymerization.

6. Biotinylated-CaM was used as a probe in conjunction with avidin-horseradish peroxidase (HRP) and chloronaphthol to identify CaM-binding proteins in Western blots of purified bovine brain MT proteins. MAP 2, tubulin, and the tau protein region are labelled by the biotinylated-CaM probe in the presence of Ca^{2+} or Cd^{2+}. This labelling is prevented by excess EGTA. These results indicate that Cd^{2+} can substitute for Ca^{2+} in supporting the binding of CaM to tubulin and MAPs in Western blots of MT proteins, and suggest that the enhanced inhibition of MT polymerization <u>in vitro</u> by Cd^{2+} in the presence of CaM is due to the Cd^{2+}-dependent binding of CaM to tubulin and MAPs.

Equipment Inventory

1. Major Equipment: none purchased.

Final Inventions Statement

1. No inventions were conceived under this grant.

Publications

Perrino, B.A. and Chou, I.N. 1986. Role of calmodulin in cadmium-induced microtubule disassembly. Cell Biol. Int. Rep. 10:565-573

Perrino. B.A. and Chou, I.N. 1987. Cytoskeletal injury resulting from the interaction of calmodulin with metal compounds. Proceedings of the Sixth International Conference on Heavy Metals in the Environment, Sept. 15-18, 1987, New Orleans. Vol. 1:332-336. This paper was presented as the Keynote Address of the Session on Health Effects: Cadmium and Mercury, Sept. 16, 1987

Perrino, B.A. and Chou, I.N. 1989. Calmodulin modulation of adverse effects of Cd^{2+} on microtubules and tubulin polymerization in vitro. Toxicology In Vitro (in press)

Perrino, B.A. and Chou, I.N. Inhibition of microtubule reassembly by Cd^{2+} and Cd^{2+}-dependent binding of calmodulin to mirotubule-associated proteins (MAPs) and tubulin. (manuscript in preparation).

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16. Abstract (Limit: 200 words): The major aim of this study was to determine whether metallothionine (MT) disassembly and inhibition of assembly caused by cadmium (7440439), mercury (7439976), and lead (7439921) in cultured 3T3 cells was due to binding to tubulin sulfhydryl groups or binding to and activation of CaM. Methylmercury was used as a model for adverse effects to MT due to tubulin sulfhydryl binding in the extracted cytoskeleton model system and during the polymerization of purified MT proteins in-vitro. The results indicated that, like calcium, the inhibitory effect of cadmium on the polymerization of purified MT proteins containing tubulin and microtubule associated proteins was enhanced by exogenously added CaM, suggesting that microtubule associated proteins were involved in mediating the sensitivity of MT to cadmium activated CaM. The findings that cadmium, like calcium, can support the binding of CaM to tubulin and MAPs in Western blots of purified MT proteins support the proposal that MAPs are important in the increased sensitivity of MT to cadmium activated CaM. The stimulation by cadmium or calcium/CaM dependent protein-kinase-II, resulting in MAP phosphorylation and MT disassembly and inhibition of assembly was not investigated.

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