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Review Article

MOLECULAR MECHANISMS OF DIKETONE NEUROTOXICITY

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SUMMARY

The important industrial and commercial solvents *n*-hexane and methyl *n*-butyl ketone undergo metabolic conversion in experimental animals and man to the neurotoxic γ-diketone 2,5-hexanedione. Several molecular mechanisms of action have been proposed to explain the pathogenesis of γ-diketone neuropathy. Such a mechanism must account for the target organ specificity, neurofilament accumulation, structure/activity relationships, in vivo covalent binding, and apparent direct axonal toxicity encountered in this syndrome. It has been proposed that the γ-diketones exert their effects by reaction with sulfhydryl moieties of energy-producing axonal glycolytic enzymes, with resultant disruption of axoplasmic transport. Others have suggested that reaction instead occurs with lysine moieties of axonal cytoskeletal proteins to form alkyl pyrrole adducts, leading to damaging physicochemical changes in these proteins. Additional hypotheses involve inhibition of axonal sterologenesis, alterations in nerve membrane properties, and reduced neurofilament proteolysis within the nerve terminal.

Although a comprehensive mechanism of action for the γ-diketones remains to be demonstrated, much progress has been made toward this goal. Ultimate success awaits elucidation of the interactions of the neurotoxic diketones with axonal components at the molecular level. Previous reviews have addressed the historical, pharmacokinetic, and neuropathological aspects of this neuropathy. The present critique will examine proposed molecular mechanisms for the γ-diketones with regard to theoretical considerations and experimental evidence.

Key words: Axonal cytoskeleton — γ-Diketone neurotoxicity — Distal axonopathy — 2,5-Hexanedione neurotoxicity — Neurofilament cross-linking — Neurofilamentous neuropathy — Pyrrole adduct formation

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INTRODUCTION

The initial reports of the neurotoxic effects of the 'hexacarbon' solvents *n*-hexane and methyl *n*-butyl ketone (MnBK) [1,2] and of their γ-diketone metabolite 2,5-hexanedione (2,5-HD) [3] led to a major experimental effort to explore the biochemical and pathological characteristics of this syndrome. Such studies have been prompted not only because of the great potential for human exposure to these solvents [4] but also because of the possibility of demonstrating a common mechanism of action for these and other chemicals causing a similar neuropathy. This type of neuropathy has been named 'central-peripheral distal axonopathy' (CPDA) based on its most distinctive neuropathological features [5]. In addition, the abnormal accumulation of neurofilaments induced by the neurotoxic γ-diketones [6,7] is not unlike that seen in certain naturally-occurring disorders, e.g. childhood giant axonal neuropathy [8]. These chemicals may therefore be useful tools for exploring the function of axonal cytoskeletal elements in normal and disease states.

An acceptable molecular mechanism for diketone neurotoxicity must account for several characteristic observations: (i) The neurotoxic diketones exhibit an organ specific toxicity since only the nervous system and testicular germinal epithelium appear to be affected, even at high levels of exposure. (ii) Only those compounds with a γ-diketone structure (i.e. two carbon spacing between the carbonyl groups), or which can be metabolized to a γ-diketone, are capable of inducing the neurotoxic syndrome [9—12]. (iii) A pathological hallmark of the neuropathy is axonal swelling due to massive accumulation of 10 nm neurofilaments within the axoplasm of selected peripheral and central nervous system fibers [6]. The distribution of the swellings (i.e. distal vs. proximal axon) appears dependent upon both the structural characteristics and administered dose of the γ-diketone [13]. (iv) MnBK and 2,5-HD react covalently with tissue macromolecules in vivo [14,15]. (v) The γ-diketones appear to act directly upon one or more axonal components, since NF accumulation can be induced by direct application of 2,5-HD to nerve fibers [16], by intraneural injection of the compound [17,18], or by its incorporation into the nutrient medium of nervous tissue culture systems [19].

Although a satisfactory mechanism has not yet been demonstrated, substantial progress has been made toward elucidation of the crucial events leading to neuropathy. Ultimate success awaits further exploration of the molecular interactions of the neurotoxic diketones with axonal components in vivo. While several comprehensive reviews [20—22] have summarized the historical development and the neuropathological and pharmacokinetic aspects of this neuropathy, the proposed molecular mechanisms of action have only been briefly discussed. The present review will critically analyze these mechanisms in view of both theoretical considerations and experimental evidence.

PROPOSED MECHANISMS OF ACTION

Inhibition of axonal glycolysis

Spencer and Schaumburg [23] initially suggested that nerve degeneration in γ-diketone neuropathy might be related to inhibition of axonal glycolytic enzymes, particularly glyceraldehyde 3-phosphate dehydrogenase (GAPDH). This hypothesis was based upon earlier work demonstrating that inhibition of GAPDH by iodoacetate resulted in decreased ATP levels and a reduction in fast axoplasmic transport within affected nerve fibers [24,25]. The inhibition was due to irreversible covalent reaction with active-site sulfhydryl groups. The glycolytic enzyme lactate dehydrogenase (LDH), which contains no active-site sulfhydryl, was not inhibited.

In order to test the hypothesis that MnBK and/or 2,5-HD might act via a similar mechanism, Sabri et al. [26] assayed GAPDH and LDH after in vitro incubation with these compounds and with non-neurotoxic 1,6-hexanediol and acetone. Inhibition of GAPDH but not LDH was observed with MnBK and 2,5-HD, while the non-neurotoxic compounds were without effect. Pre-incubation of enzyme with neurotoxin was necessary for inhibition, and the prior addition of dithiothreitol (DTT) protected the enzyme from inactivation. Similar effects were observed with whole rat sciatic nerve and with rat brain homogenate exposed in vitro to MnBK or 2,5-HD [26]. In vitro inhibition of the major glycolytic enzyme phosphofructokinase (PFK) by 2,5-HD but not by the non-neurotoxic β-diketone 2,4-HD was also revealed [27]. A 31% decrease in PFK activity was reported in brain homogenates from rats receiving 0.5% 2,5-HD in the drinking water for 10—12 weeks, at which time the animals were severely paralyzed [27]. It was not reported whether this value represented a statistically significant reduction. The authors concluded that both MnBK and 2,5-HD react irreversibly with sulfhydryl moieties of GAPDH and PFK to render the enzymes inactive.

Related studies had revealed an apparent blockade of fast anterograde axonal transport through regions of NF swellings following administration to rats of either MnBK [28] or 2,5-hexanediol [29] (both metabolized to 2,5-HD). Based on these and the previously described results, Spencer et al. [30] postulated that the reduction in energy supplies resulting from glycolytic enzyme inhibition would produce a disruption of both fast and slow axonal transport which would be particularly acute at regions of high energy demand, i.e. nodes of Ranvier. This compromised transport system would presumably only be able to supply replacement enzymes to the more proximal area of the axon at the expense of distal regions. In addition, slowly transported neurofilaments would begin to accumulate at nodes of Ranvier, further reducing nutrient transport. The result would be Wallerian-type nerve degeneration in areas distal to the NF accumulations.

Several in vitro studies have been performed to test this hypothesis.

Howland et al. [31] reported the inhibition of enolase, another glycolytic sulfhydryl enzyme, after incubation with 2,5-HD. The inhibition differed from that of GAPDH and PFK since preincubation was not required and addition of DTT potentiated the inhibition. The authors speculated that the target organ specificity and lack of systemic effects of the γ-diketones might be due to selective inhibition of a particular neuronal isoenzyme of enolase. Another study examined the in vitro effects of 2,5-HD on the non-glycolytic enzymes transketolase and succinate dehydrogenase, neither of which were inhibited by the neurotoxin [32]. A dose-related decrease in ATP levels in cat sciatic nerves treated in vitro with 2,5-HD was also observed, although relatively high concentrations (>50 mM) of the compound were required to produce this effect. ATP levels could be restored by addition of sodium pyruvate (the end product of glycolysis) to the incubation mixture. Non-neurotoxic 1,6-hexanediol had no effect on ATP levels. In vitro inhibition of GAPDH after prolonged exposure to lower levels (1—5 mM) of 2,5-HD has also been reported [33].

Other studies have attempted to detect significant alterations in glycolysis after in vivo exposure to 2,5-HD, a task which is complicated by the extreme difficulty in selectively measuring such changes in the axonal compartment. Couri and Nachtman [34] demonstrated a decreased oxygen uptake in sciatic nerve from rats receiving 0.1 or 0.5% 2,5-HD in the drinking water. These data were cited [22,31] as evidence for an inhibition of axonal glycolysis, although the decrease in oxygen uptake did not appear to be dose-related or correlated with clinical signs of neuropathy. Griffiths et al. [35] reported a decreased local glucose utilization in the superior colliculus of rats after 3 weeks exposure to 0.5% 2,5-HD in the drinking water, prior to the onset of clinically observable neuropathy. This effect was suggested to reflect a lower energy demand due to impaired impulse conduction, rather than a primary disruption in energy supplies. Glycolytic enzyme activities were not reported in the study. Anderson and Dunham [36] showed that i.p. administration of 35 daily doses of 0.28 g 2,5-HD/kg to rats resulted in impaired sciatic nerve membrane excitability, prior to clinical or histopathological signs of neuropathy. They suggested that this finding was consistent with altered energy utilization in the nerve. LoPachin et al. [37] demonstrated no alteration in K and only a slight decrease in V_{max} for glucose-dependent lactate production in brain and spinal cord from rats severely intoxicated with 2,5-HD. Sabri [33] reported that GAPDH activities were decreased by approx. 25% in sciatic nerve, but not in brain or liver, from rats receiving 0.5% 2,5-HD in the drinking water for 12 weeks. Severe paralysis and body weight loss were evident as a result of this dosing regimen.

In a related series of studies, Gillies et al. [38] examined the incorporation of label from [^{14}C]glucose into sciatic nerve lipids from 2,5-HD-treated rats. The authors reasoned that inhibition of GAPDH should result in a decreased supply of glycerol-3-phosphate and acetyl-CoA for lipogenesis. Synthesis of sterols was found to be depressed, while that of free fatty acids, triacylglycerols, and phospholipids was not different from that of pair-fed control

animals. They concluded that while glycolysis was not inhibited in vivo, some defect (as discussed below) in the lipogenic pathways leading from acetyl-CoA to sterols was present in γ-diketone-exposed animals.

Although there is some evidence for axonal glycolytic inhibition after 2,5-HD exposure, there are substantial objections to a primary mechanism involving energy disruption in γ-diketone neuropathy. In vitro inhibition of GAPDH and PFK requires relatively high concentrations of 2,5-HD and/or prolonged incubation times, with diketone:protein molar ratios approaching 10^4 [26,27]. Such concentrations are not encountered in nervous tissue after systemic exposure to *n*-hexane, where conversion to 2,5-HD is relatively slow [39]. Although some enzyme inactivation by low levels of metabolically-derived 2,5-HD may occur in vivo, it is unknown whether such inactivation could outpace enzyme replacement. Conclusive evidence of in vivo glycolytic enzyme inhibition during 2,5-HD intoxication is lacking, with only moderate inhibition of brain PFK [27] and spinal cord GAPDH [33] reported in rats with severe paralysis and significant body weight loss. Other reported [34,35] effects of 2,5-HD exposure (i.e. decreased glucose utilization and O_2 uptake) may not necessarily reflect a specific inhibition of glycolysis within the axon.

It is also difficult to account for the characteristic NF accumulation in nerve fibers following either direct or systemic exposure to 2,5-HD by inhibition of axonal glycolysis. Although it has been shown that fast transport can be inhibited by disruption of glycolysis and lack of ATP [24,25], a similar energy requirement has not been demonstrated for the movement of neurofilaments in slow axonal transport. NF transport within the normal axon may, in fact, be dependent upon microtubule (MT) movement [40], which does not appear to be altered during γ-diketone neuropathy [28]. It is presently unclear whether NF transport is slowed [41] or accelerated [42] during γ-diketone exposure. A recent extension [43] of the glycolytic inhibition hypothesis proposes that metabolic energy is required for normal NF-MT interactions and that disruption of this energy supply will result in dissociation of these cytoskeletal elements. Current evidence suggests that NF-MT organization may depend upon physicochemical interactions between NF subunit and microtubule-associated proteins, rather than an active, energy-dependent mechanism [44]. Such interactions can be reproduced in the absence of energy-generating systems. As discussed below, the rapid reorganization of axonal cytoskeletal elements following intraneural injection of 2,5-HD [17,18] may instead be more consistent with direct covalent interaction of the compound with these elements. Resolution of this question awaits studies on the spatial and temporal changes in energy status along individual nerve fibers during γ-diketone exposure.

Physicochemical alteration of neurofilaments

In 1980 it was suggested independently by two laboratories that the critical in vivo reaction site of 2,5-HD with protein was the lysine ε-amine function rather than the cysteine sulfhydryl moiety [45,46]. Both groups

demonstrated that in vitro incubation of 2,5-HD with primary amines resulted in a progressive irreversible reaction and the appearance of an orange chromophore in the incubation mixture. Based on an analogous reaction of malonaldehyde with amino acids [47], Graham [48] proposed the formation of fluorescent 'conjugated Schiff bases' as products of this reaction. The non-neurotoxicity of the β-diketones was attributed to their relatively low water solubility and to their propensity for dimerization via hydrogen bonding. DeCaprio and Weber [45,49] suggested instead that the reaction produced a 2,5-dimethylpyrrole moiety which could undergo complex secondary oxidative reactions to yield chromophoric polymers. This hypothesis was based upon well-known synthetic routes for the 1,2,5-trisubstituted pyrroles and other aspects of pyrrole chemistry [50—52]. Such a reaction would account for the structure/activity relationships in diketone neuropathy, since only γ-diketones could form the pyrrole ring.

Subsequent mass spectral and IR analysis confirmed the reaction product of the lysine ε-amine group and 2,5-HD as the substituted pyrrole derivative ε-*N*-(2,5-dimethylpyrrolyl)norleucine (2,5-DMPN) rather than a Schiff base [15]. The pyrrole structure of the 2,5-HD and ethanolamine reaction product was similarly confirmed [53]. Incubation of other γ-diketones (2,5-heptanedione, 3,6-octanedione) with amines yielded pyrrole products which became chromophoric with time. Gel electrophoretic analysis of proteins treated in vitro with 2,5-HD (diketone/lysine molar ratios of 100—1000:1) revealed progressive loss of lysine, formation of pyrrole adducts, oxidation (appearance of color), and apparent polymerization [15,53]. HPLC analysis demonstrated the presence of the 2,5-DMPN residue in serum protein hydrolysates from hens receiving 200 mg 2,5-HD/kg/day for 2 weeks [15]. Widespread adduct formation was seen in brain, liver, and kidney protein from these animals [54]. Clearance of the adduct from tissue protein after cessation of exposure was also observed.

Other studies have examined the neurotoxicity and amine reactivity of γ-diketones structurally related to 2,5-HD. Animals receiving 3,4-dimethyl-2,5-HD (DHMD) rapidly developed a neuropathy characterized by NF accumulations located in proximal areas of axons as opposed to the distal swellings encountered with 2,5-HD [13]. Reaction of this derivative with lysine ε-amine groups would produce a 2,3,4,5-tetramethyl pyrrole adduct, which was subsequently detected in 'axonal pad' proteins from rats receiving 0.1% DMHD in the drinking water for 21 days [55]. Apparent cross-linking of the erythrocyte membrane protein spectrin was also observed in these animals. In vitro studies revealed that both pyrrole formation and secondary cross-linking were accelerated with DMHD as compared with 2,5-HD [55]. A recent study reported that administration of 3-methyl-2,5-hexanedione to rats induced NF accumulations localized to the midportion of susceptible nerve fibers [56].

The phenomenon of pyrrole adduct formation in γ-diketone neuropathy has led to several mechanistic hypotheses based upon covalent modification of NF or other axonal cytoskeletal proteins. The early observations of

covalent cross-linking during in vitro protein/2,5-HD incubation [45,48] led to the suggestion that such cross-linking might underlie the NF accumulation in this neuropathy. Graham initially proposed that cross-linking was the result of conjugated Schiff base formation [48], a mechanism that was later modified in favor of one involving secondary autoxidation of pyrrole adducts [53]. Such altered neurofilaments would conceivably be unable to pass through areas of restricted axonal calibre (i.e. nodes of Ranvier) and would accumulate on the proximal side of these restrictions. Since no significant turnover of neurofilaments is believed to occur within the axon itself [57], such damage would be cumulative. NF accumulation would ultimately lead to a blockade of nutrient flow into the distal axon and nerve degeneration.

Evidence suggests that covalent cross-linking of NF proteins probably does occur during γ-diketone exposure, although the actual chemical species involved in this process are not known. In vitro treatment of rat sciatic nerve with [^{14}C]DMHD resulted in the formation of radiolabeled protein polymers as detected by increased retention on nitrocellulose filters [58]. Similar exposure of nerves, in which the NF protein had been previously labeled with [^{35}S]methionine, to unlabeled DMHD revealed that much of the polymeric material had been derived from these proteins [58]. In vivo formation of pyrrole adducts, new protein bands, and high-mol. wt. polymers has been observed in axonal cytoskeletal protein preparations from rats treated with 2,5-HD [59].

Although a mechanism of covalent cross-linking of NF protein can account for many aspects of γ-diketone neuropathy, it may not be sufficient to explain the rapid reorganization of neurofilaments and microtubules following direct intraneural injection of these compounds [17,18]. While substantial pyrrole formation would have occurred under these conditions, adequate time would probably not have elapsed to allow significant secondary cross-linking between these adducts [15]. It has also been argued that the observed differences in the proximo-distal distribution of axonal swellings produced by 2,5-HD and DMHD are the result of a more rapid cross-linking of the adduct formed by the latter compound [13,55]. If this factor is a major determinant of the proximo-distal distribution of NF swellings, then a clear dose/response relationship should exist for this parameter with 2,5-HD exposure. This has not been demonstrated in 2,5-HD neuropathy, although decreasing the dose of DMHD does appear to produce more distally-located swellings [13]. A related observation is the similar time frame (and effective dose) for development of distal sciatic nerve NF swellings with 2,5-HD in both the rat and the chicken [54,60]. Since the sciatic nerve in the latter species is several times longer, one would expect more proximally-oriented swellings given equal exposure to the diketone and an equal rate of NF transport. Finally, administration to rats of glutaraldehyde, a demonstrated protein cross-linking agent, does not result in NF accumulation or nerve degeneration [12].

As an alternative to a mechanism involving covalent cross-linking, NF

accumulation may instead be due to increased hydrophobic interactions and/or changes in the tertiary structure of the proteins comprising these cytoskeletal elements [15,54]. The NF proteins are relatively insoluble [61] and thus the formation of even a few uncharged, hydrophobic pyrrole residues per molecule of protein might be sufficient to induce aggregation. The relatively greater neurotoxic potency of DMHD might be related to either the extreme hydrophobicity of the 2,3,4-5-tetramethylpyrrole adduct formed by this compound or to a potentially enhanced ability of DMHD to penetrate the axon as compared with 2,5-HD. Recent results suggest that the average level of lysine conversion in axonal proteins from rats exposed to 2,5-HD may be quite low; on the order of 1—2 adducts/molecule [59]. While such low absolute pyrrole levels are probably not consistent with major increases in protein hydrophobicity, it may be that adduct levels in specific NF subunit proteins are considerably higher.

Another possibility is that γ-diketone binding results in the loss of specific lysine ϵ-amine groups that are essential to either the NF transport process or to normal interaction between these structures and other axonal components [17]. Evidence for such a mechanism derives from the previously discussed studies describing direct intraneural injection of 2,5-HD and the subsequent rapid reorganization of neurofilaments and microtubules into patterns similar to those induced by systemic intoxication [17,18]. This observation suggests that the initial pyrrole formation leads to an immediate rearrangement of cytoskeletal components within the axon. Although the mechanism of NF transport within the axon is not presently known, intimate association of filaments with microtubules is believed to be essential [40,62]. A disruption of NF-NF and/or NF-MT interactions might ultimately result in neurofilaments being 'left behind', while MT transport proceeded unabated. These interactions are believed to be mediated by the carboxy-terminal 'tail' region of the high-molecular weight NF subunit protein (NF-H), a region which is particularly rich in lysine residues (~20 mol%) [63,64]. It is tempting to speculate that modification of specific lysine residues within this region by 2,5-HD might lead to disruption of NF-MT interactions, segregation of neurofilaments within the axoplasm, and failure of NF transport. Although covalent cross-linking between neurofilaments might still eventually occur, it would be incidental to the neurotoxic mechanism. A recent report [59] describes a time-dependent decrease in relative levels of the NF-H subunit protein in rat brain stem following exposure to 0.5% 2,5-HD in the drinking water for up to 8 weeks.

Inhibition of sterologenesis

Gillies et al. [65] reported a decreased incorporation of [1-^{14}C]acetate into triacylglycerols, sterols, squalene, and ubiquinone in sciatic nerves of rats receiving 1% 2,5-HD in the drinking water for 6 weeks. Additional studies with [^{14}C]acetate and [^{3}H]mevalonolactone revealed a block in the pathway of ubiquinone synthesis between acetate and mevalonate, possibly at the level of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-

CoA reductase) [66]. The use of [^{14}C]leucine confirmed a specific inhibition of HMG-CoA reductase by 2,5-HD [67]. The authors concluded that the inhibition of ubiquinone biosynthesis might contribute to 2,5-HD neuropathy via disruption of oxidative phosphorylation within the nerve.

It is difficult to account for all of the characteristics of γ-diketone neuropathy by a primary mechanism of HMG-CoA reductase inhibition and depletion of ubiquinone. Sterologenesis from [^{14}C]acetate was not affected when rat sciatic nerves were incubated in vitro with 1 mM 2,5-HD, suggesting that the effect on lipogenesis was not due to a direct interaction of the compound with axonal components [66]. In addition, Spencer et al. [68] demonstrated that administration of sodium dichloroacetate, a known inhibitor of HMG CoA-reductase, did not produce a neuropathy similar to that from 2,5-HD. It may be that this phenomenon represents a secondary response of the nerve fiber rather than a primary mechanism of neuropathy, although it may be important in the testicular atrophy caused by 2,5-HD [67].

OTHER PROPOSED MECHANISMS

Couri and Nachtman [34] reported biophysical changes in sciatic nerve homogenates and purified myelin preparations from rats receiving 0.1% or 0.5% 2,5-HD in the drinking water for 2 months. Arrhenius plots revealed a lack of normal temperature phase transitions in nerve homogenates from treated animals, and these changes were attributed to alterations in myelin lipid bilayers. Myelin membrane microviscosity was also decreased in animals at the 0.5% 2,5-HD level. The authors concluded that the membrane effects were not attributable to direct interaction of the diketone with membrane components, since addition of 2,5-HD to control nerve homogenates could not reproduce the changes. Clinical signs of neuropathy were not observed in animals at the 0.1% dose level, suggesting that the membrane effects preceded pathological changes in the nerve fiber. Additional studies revealed that similar alterations were present in red cell membranes from 2,5-HD-treated rats, and that such changes resembled those seen in myelin membranes during Wallerian degeneration following nerve section [69]. It has been proposed that these membrane effects may be related to either the pathogenesis of myelin changes or to the mechanism of axonal degeneration in γ-diketone neuropathy [22].

Cavanagh and Bennetts [70] suggested that γ-diketone neuropathy might be due to a specific inhibition of axonal calcium-activated proteases believed responsible for normal degradation of NF protein. These proteases are localized in the nerve terminals, and can be inhibited by sulfhydryl reagents such as iodoacetic acid [57,71]. The authors speculated that reaction of 2,5-HD with critical sulfhydryl moieties of the protease might render the enzyme inactive and allow neurofilaments to accumulate in the distal axon. Such a hypothesis is difficult to reconcile with the observed distal but non-terminal localization of NF accumulations. Inhibition of

the protease would presumably result in an initial accumulation of neurofilaments in the nerve terminals, an effect which has not been reported in 2,5-HD neuropathy. In addition, NF masses which reach the nerve terminal during neuropathy appear to undergo normal processing [72].

Other mechanisms have been proposed without any direct experimental evidence. Based on structural similarities to the potassium ionophore valinomycin, it was suggested that 2,5-HD might act to disrupt axonal membrane ion balance (cited in Ref. 20). Schoental and Cavanagh [73] speculated that the γ-diketones might simultaneously react with a sulfhydryl group of a thiamine-dependent enzyme within the axon (such as pyruvate decarboxylase) and with the amino group of thiamine itself. The covalent bridge thus formed would presumably destroy the catalytic activity of the enzyme. Other proposed mechanisms include chelation of mitochondrial calcium and acetylcholinesterase inhibition [20]. None of these hypotheses can account for all of the pathological and biochemical characteristics of γ-diketone neuropathy.

CONCLUSIONS

Although substantial progress has been made since the initial reports of n-hexane and MnBK neurotoxicity, the molecular mechanism of action of these compounds remains elusive. Experimental evidence suggests that the mechanism almost certainly involves covalent interaction of 2,5-HD with lysine amine groups of NF and/or other axonal proteins. Qualitative and quantitative evaluation of this binding during in vivo exposure to 2,5-HD is a prerequisite to further progress in this area. Another critical question is whether secondary autoxidation or cross-linking of pyrrole adducts is a requirement for neurotoxicity, or whether pyrrole formation alone is sufficient.

The status of energy production and usage along the nerve fiber also requires further study, since several lines of evidence indicate that this parameter may be altered in γ-diketone neuropathy. It is currently unclear whether the observed effects represent a primary mechanism of action or a secondary response of the nerve. Although a widespread reduction in axonal glycolysis during 2,5-HD intoxication appears unlikely, local disruptions in energy supply might have profound effects upon nutrient and/or NF transport in the axon. Another related problem concerns the identification of the major sites of entry of γ-diketones into the nerve cell (i.e. via perikaryon, node of Ranvier, nerve terminal, or indirectly through glial cells?). Detection of such regional variations in energy production or γ-diketone uptake represents a significant technical challenge.

It is likely that experiments of this nature will serve to illuminate many aspects of γ-diketone neuropathy and would allow progress toward evaluating the common or 'unifying' mechanisms proposed [17,30,43,74] for these and other similar neurotoxins (e.g. acrylamide, carbon disulfide, β,β'-iminodipropionitrile). The difficulty in achieving this goal is apparent

if one considers the diverse chemical structures of these compounds and the differences in proximo-distal and CNS vs. PNS distribution of the induced NF accumulations. Although such a common mechanism may ultimately prove to be unsupportable, it nevertheless serves as a useful working hypothesis in developing additional data on these important neurotoxic chemicals.

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REFERENCES

1 S. Yamada, Intoxication polyneuritis in the workers exposed to n-hexane. Japan. J. Ind. Health, 9 (1967) 651.
2 N. Allen, J.R. Mendell, D.J. Billmaier, R.E. Fontaine and J. O'Neill, Toxic polyneuropathy due to methyl n-butyl ketone: an industrial outbreak, Arch. Neurol., 32 (1975) 209.
3 P.S. Spencer and H.H. Schaumburg, Experimental neuropathy produced by 2,5-hexanedione — A major metabolite of the neurotoxic industrial solvent methyl n-butyl ketone, J. Neurol. Neurosurg. Psych., 38 (1975) 771.
4 NIOSH, Occupational exposure to alkanes (C5-C8). DHEW Publication No. 77-151. Washington, D.C.: Government Publication Office, 1977.
5 P.S. Spencer and H.H. Schaumburg, Central-peripheral distal axonopathy — The pathology of dying-back polyneuropathies, in: H. Zimmerman (Ed.), Progress in Neuropathology, Vol. 3. Grune and Stratton, New York, 1976, pp. 253—95.
6 P.S. Spencer and H.H. Schaumburg, Ultrastructural studies of the dying-back process. III. The evolution of experimental peripheral giant axonal degeneration, J. Neuropathol. Exp. Neurol., 36 (1977) 276.
7 P.S. Spencer and H.H. Schaumburg, Ultrastructural studies of the dying-back process. IV. Differential vulnerability of PNS and CNS fibers in experimental central-peripheral distal axonopathies, J. Neuropathol. Exp. Neurol., 36 (1977) 300.
8 S.D.J. Pena, Giant axonal neuropathy: an inborn error of organization of intermediate filaments, Muscle Nerve, 5 (1982) 166.
9 M.S. Abdel-Rahman, L.B. Hetland and D. Couri, Toxicity and metabolism of methyl n-butyl ketone, Am. Ind. Hyg. Assoc. J., 37 (1976) 95.
10 D. Couri, M.S. Abdel-Rahman and L.B. Hetland, Biotransformation of n-hexane and methyl n-butyl ketone in guinea pigs and mice, Am. Ind. Hyg. Assoc. J., 39 (1978) 295.
11 G.D. DiVincenzo, C.J. Kaplan and J. Dedinas, Characterization of the metabolites of methyl n-butyl ketone, methyl iso-butyl ketone, and methyl ethyl ketone in guinea pig serum and their clearance, Toxicol. Appl. Pharmacol., 36 (1976) 511.
12 P.S. Spencer, M.C. Bischoff and H.H. Schaumburg, On the specific molecular configuration of neurotoxic alipathic hexacarbon compounds causing central-peripheral distal axonopathy, Toxicol. Appl. Pharmacol., 44 (1978) 17.
13 D.C. Anthony, K. Boekelheide and D.G. Graham, The effect of 3,4-dimethyl substitution on the neurotoxicity of 2,5-hexanedione. I. Accelerated clinical neuropathy is accompanied by more proximal axonal swellings, Toxicol. Appl. Pharmacol., 71 (1983) 362.
14 G.D. DiVincenzo, M.L. Hamilton, C.J. Kaplan and J. Dedinas, Metabolic fate and disposition of ^{14}C-labelled methyl n-butyl ketone in the rat, Toxicol. Appl. Pharmacol., 41 (1977) 547.

15 A.P. DeCaprio, E.J. Olajos and P. Weber, Covalent binding of a neurotoxic n-hexane metabolite: conversion of primary amines to substituted pyrrole adducts by 2,5-hexanedione, Toxicol. Appl. Pharmacol., 65 (1982) 440.
16 M.J. Politis, R.G. Pelligrino and P.S. Spencer, Ultrastructural studies of the dying-back process. V. Axonal neurofilaments accumulate at sites of 2,5-hexanedione application: evidence for nerve fiber dysfunction in experimental hexacarbon neuropathy, J. Neurocytol., 9 (1980) 505.
17 J.W. Griffin, K.E. Fahnestock, D.L. Price and L.C. Cork, Cytoskeletal disorganization induced by local application of β,β'-iminodipropionitrile and 2,5-hexanedione, Ann. Neurol., 14 (1983) 55.
18 J.C. Zagoren, M.J. Politis and P.S. Spencer, Rapid reorganization of the axonal cytoskeleton induced by a gamma-diketone, Brain Res., 270 (1983) 162.
19 B. Veronesi, E.R. Peterson, M.B. Bornstein and P.S. Spencer, Ultrastructural studies of the dying-back process. VI. Examination of nerve fibers undergoing giant axonal degeneration in organotypic culture, J. Neuropathol. Exp. Neurol., 42 (1983) 153.
20 P.S. Spencer, H.H. Schaumburg, M.I. Sabri and B. Veronesi, The enlarging view of hexacarbon neurotoxicity, CRC Crit. Rev. Toxicol., 4 (1980) 279.
21 J.W. Griffin, Hexacarbon neurotoxicity, Neurobehav. Toxicol. Teratol., 3 (1981) 437.
22 D. Couri and M. Milks, Toxicity and metabolism of the neurotoxic hexacarbons n-hexane, 2-hexanone, and 2,5-hexanedione, Annu. Rev. Pharmacol. Toxicol., 22 (1982) 145.
23 P.S. Spencer and H.H. Schaumburg, Pathobiology of neurotoxic axonal degeneration, in: S.G. Waxman (Ed.), Physiology and Pathobiology of Axons, Raven Press, New York, 1978, pp. 265—82.
24 M.I. Sabri and S. Ochs, Inhibition of glyceraldehyde-3-phosphate dehydrogenase in mammalian nerve by iodoacetic acid, J. Neurochem., 18 (1971) 1509.
25 M.I. Sabri and S. Ochs, Relation of ATP and creatine phosphate to fast axoplasmic transport in mammalian nerve, J. Neurochem., 19 (1972) 2821.
26 M.I. Sabri, C.L. Moore and P.S. Spencer, Studies on the biochemical basis of distal axonopathies. I. Inhibition of glycolysis by neurotoxic hexacarbon compounds, J. Neurochem., 32 (1979) 683.
27 M.I. Sabri, K. Ederle, C.E. Holdsworth and P.S. Spencer, Studies on the biochemical basis of distal axonopathies. II. Specific inhibition of fructose-6-phosphate kinase by 2,5-hexanedione and methyl butyl ketone, Neurotoxicology, 1 (1979) 285.
28 J.R. Mendell, Z. Sahenk, K. Saida, H.S. Weiss, R. Savage and D. Couri, Alterations of fast axoplasmic transport in experimental methyl n-butyl ketone neuropathy, Brain Res., 133 (1977) 107.
29 J.W. Griffin, D.L. Price and P.S. Spencer, Fast axonal transport through giant axonal swellings in hexacarbon neuropathy, J. Neuropathol. Exp. Neurol., 36 (1977) 603.
30 P.S. Spencer, M.I. Sabri, H.H. Schaumburg and C.L. Moore, Does a defect of energy metabolism in the nerve fiber underlie axonal degeneration in polyneuropathies? Ann. Neurol., 5 (1979) 501.
31 R.D. Howland, I.L. Vyas, H.E. Lowndes and T.M. Argentiers, The etiology of toxic peripheral neuropathies: in vitro effects of acrylamide and 2,5-hexanedione on brain enolase and other glycolytic enzymes, Brain Res., 202 (1980) 131.
32 M.I. Sabri, In vitro effect of n-hexane and its metabolites on selected enzymes in glycolysis, pentose phosphate pathway and citric acid cycle, Brain Res., 297 (1984) 145.
33 M.I. Sabri, Further observations on in vitro and in vivo effects of 2,5-hexanedione on glyceraldehyde-3-phosphate dehydrogenase, Arch. Toxicol., 55 (1984) 191.
34 D. Couri and J.P. Nachtman, Biochemical and biophysical studies of 2,5-hexanedione neuropathy, Neurotoxicology, 1 (1979) 269.
35 I.R. Griffiths, P.A.T. Kelly, S. Carmichael, M. McCulloch and M. Waterston, The relationship of glucose utilization and morphological change in the visual system in hexacarbon neuropathy, Brain Res., 222 (1981) 447.

36 R.J. Anderson and C.B. Dunham, Electrophysiologic deficits in peripheral nerve as a discriminator of early hexacarbon neurotoxicity, J. Toxicol. Environ. Health, 13 (1984) 835.
37 R.M. LoPachin, R.W. Moore, L.A. Menahan and R.E. Peterson, Glucose-dependent lactate production by homogenates of neuronal tissues prepared from rats treated with 2,4-dithiobiuret, acrylamide, p-bromophenylacetylurea, and 2,5-hexanedione, Neurotoxicology, 5 (1984) 25.
38 P.J. Gillies, R.M. Norton and J. Bus, Inhibition of sterologenesis but not glycolysis in 2,5-hexanedione-induced distal axonopathy in the rat, Toxicol. Appl. Pharmacol., 59 (1981) 287.
39 W.J. Krasavage, J.L. O'Donoghue, G.D. DiVincenzo and C.J. Terhaar, The relative neurotoxicity of methyl n-butyl ketone, n-hexane and their metabolites, Toxicol. Appl. Pharmacol., 52 (1980) 433.
40 R.J. Lasek, M.M. Oblinger and P.F. Drake, Molecular biology of neuronal geometry: expression of neurofilament genes influences axonal diameter, Cold Spring Harbor Symp. Quant. Biol., 48 (1983) 731.
41 J.W. Griffin, D.C. Anthony, K.E. Fahnestock, P.N. Hoffman and D.G. Graham, 3,4-Dimethyl-2,5-hexanedione impairs the axonal transport of neurofilament proteins, J. Neurosci., 4 (1984) 1516.
42 S. Monaco, L. Autilio-Gambetti, D. Zabel and P. Gambetti, Giant axonal neuropathy: acceleration of neurofilament transport in optic axons, Proc. Natl. Acad. Sci. U.S.A., 82 (1985) 920.
43 P.S. Spencer, M.S. Miller, S.M. Ross, B.W. Schwab and M.I. Sabri, Biochemical mechanisms underlying primary degeneration of axons, in: A. Lajtha (Ed.), Handbook of Neurochemistry, Vol. 9, Plenum Press, New York, 1985, pp. 31—65.
44 E.J. Aamodt and R.C. Williams, Microtubule-associated proteins connect microtubules and neurofilaments in vitro, Biochemistry, 23 (1984) 6023.
45 A.P. DeCaprio and P. Weber, In vitro studies on the amino group reactivity of a neurotoxic hexacarbon solvent, Pharmacologist, 22 (1980) 222.
46 D.G. Graham, M.B. Abou-Donia and B.R. Shaw, The formation of conjugated Schiff bases with 2,5-hexanedione: an hypothesis for the molecular basis of hexacarbon neuropathy, Abstracts — Society of Toxicology 19th Annual Meeting, Washington, D.C., 1980, A32.
47 K.S. Chio and A.L. Tappel, Synthesis and characterization of the fluorescent products derived from malonaldehyde and amino acids, Biochemistry, 8 (1969) 2821.
48 D.G. Graham, Hexane polyneuropathy: a proposal for the pathogenesis of a hazard of occupational exposure and inhalant abuse, Chem.-Biol. Interact., 32 (1980) 339.
49 A.P. DeCaprio and P. Weber, Conversion of lysine ϵ-amino groups to substituted pyrrole derivatives by 2,5-hexanedione: a possible mechanism of protein binding, Toxicologist, 1 (1981) 134.
50 E.S. Waight, Hydroxy-aldehydes and -ketones and related compounds; Dicarbonyl compounds, in: S. Coffey (Ed.), Rodd's Chemistry of Carbon Compounds, Vol. 1, Part D, Elsevier Publishing Co., New York, 1965, pp. 71—73.
51 J.M. Patterson, Recent synthetic methods for pyrroles and pyrrolenines (2H- or 3H-pyrroles), Synthesis, 5 (1976) 281.
52 R.A. Jones and G.P. Bean, The Chemistry of Pyrroles, Academic Press, New York, 1977.
53 D.G. Graham, D.C. Anthony, K. Boekelheide, N.A. Maschmann, R.G. Richards, J.W. Wolfram and B.R. Shaw, Studies of the molecular pathogenesis of hexane neuropathy. II. Evidence that pyrrole derivatization of lysyl residues leads to protein cross-linking, Toxicol. Appl. Pharmacol., 64 (1982) 415.
54 A.P. DeCaprio, N.L. Strominger and P. Weber, Neurotoxicity and protein binding of 2,5-hexanedione in the hen, Toxicol. Appl. Pharmacol., 68 (1983) 297.
55 D.C. Anthony, K. Boekelheide, C.W. Anderson and D.G. Graham, The effect of 3,4-dimethyl substitution on the neurotoxicity of 2,5-hexanedione. II. Dimethyl substitution accelerates pyrrole formation and protein cross-linking, Toxicol. Appl. Pharmacol., 71 (1983) 372.

56 S. Monaco, T. Wongmongkolrit, L. Sayre, L. Autilio-Gambetti and P. Gambetti, Giant axonopathy in 3-methyl-2,5-hexanedione (3-M-2,5-HD). Effect on morphology and slow axonal transport, J. Neuropathol. Exp. Neurol., 43 (1984) 304.
57 R.J. Lasek and P.N. Hoffman, The neuronal cytoskeleton, axonal transport and axonal growth, in: R. Golden, T. Pollard and J. Rosenbaum, (Eds.), Cell Motility, Cold Spring Harbor Conference on Cell Proliferation, Vol. 3, Cold Spring Harbor Laboratory, New York, 1976, pp. 1021—1049.
58 D.G. Graham, G. Szakal-Quin, J.W. Priest and D.C. Anthony, In vitro evidence that covalent crosslinking of neurofilaments occurs in γ-diketone neuropathy, Proc. Natl. Acad. Sci. U.S.A., 81 (1984) 4979.
59 A.P. DeCaprio and E.A. O'Neill, Alterations in rat axonal cytoskeletal proteins induced by in vitro and in vivo 2,5-hexanedione exposure, Toxicol. Appl. Pharmacol., 78 (1985) 235.
60 M.B. Abou-Donia, H.M. Makkawy and D. Graham, The relative neurotoxicities of n-hexane, methyl n-butyl ketone, 2,5-hexanediol, and 2,5-hexanedione following oral or intraperitoneal administration in hens, Toxicol. Appl. Pharmacol., 62 (1982) 369.
61 R. Liem, Simultaneous separation and purification of neurofilament and glial filament proteins from brain, J. Neurochem., 38 (1982) 142.
62 J.-F. Leterrier, R.K.H. Liem and M.L. Shelanski, Interactions between neurofilaments and microtubule-associated proteins: a possible mechanism for intraorganellar bridging, J. Cell Biol., 95 (1982) 982.
63 N. Geisler, E. Kaufmann, S. Fischer, U. Plessmann and P. Weber, Neurofilament architecture combines structural principles of intermediate filaments with carboxy-terminal extensions increasing in size between triplet proteins, EMBO J., 2 (1983) 1295.
64 M.H. Ellisman and K.R. Porter, Microtrabecular structure of the axoplasmic matrix: visualization of cross-linking structures and their distribution, J. Cell. Biol., 87 (1980) 464.
65 P.J. Gillies, R.M. Norton and J.S. Bus, Effect of 2,5-hexanedione on lipid biosynthesis in sciatic nerve and brain of the rat, Toxicol. Appl. Pharmacol., 54 (1980) 210.
66 P.J. Gillies, R.M. Norton, E.L. White and J.S. Bus, Inhibition of sciatic nerve sterologenesis in hexacarbon-induced distal axonopathy in the rat, Toxicol. Appl. Pharmacol., 54 (1980) 217.
67 P.J. Gillies, R.M. Norton, T.S. Baker and J.S. Bus, Altered lipid metabolism in 2,5-hexanedione-induced testicular atrophy and peripheral neuropathy in the rat, Toxicol. Appl. Pharmacol., 59 (1981) 293.
68 P.S. Spencer, M.C. Bischoff and P.W. Stacpoole, Differential neurotoxicity of dichloroacetate and 2,5-hexanedione: implication for the pathogenesis of gamma-diketone neuropathy, Toxicologist, 1 (1981) 51.
69 J.P. Nachtman and D. Couri, Biophysical and electrophysiological studies of hexanedione neurotoxicity, Neurotoxicology, 2 (1981) 541.
70 J.B. Cavanagh and J.B. Bennetts, On the pattern of changes in the rat nervous system produced by 2,5-hexanediol: a topographical study by light microscopy, Brain, 104 (1981) 297.
71 H.C. Pant and S. Gainer, Properties of calcium activated proteases in squid axoplasm which selectively degrades neurofilament protein, J. Neurobiol., 11 (1980) 1.
72 J.B. Cavanagh, The pattern of recovery of axons in the nervous system of rats following 2,5-hexanediol intoxication: a question of rheology? Neuropathol. Appl. Neurobiol., 8 (1982) 19.
73 R. Schoental and J.B. Cavanagh, Mechanisms involved in the "dying-back" process — An hypothesis implicating coenzymes, Neuropathol. Appl. Neurobiol., 3 (1977) 145.
74 D.G. Graham, D.C. Anthony and K. Boekelheide, In vitro and in vivo studies of the molecular pathogenesis of n-hexane neuropathy, Neurobehav. Toxicol. Teratol., 4 (1982) 629.