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Acrylamide-Induced Nerve Terminal Damage: Relevance to Neurotoxic and Neurodegenerative Mechanisms

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Acrylamide (ACR) has demonstrable neurotoxic effects in animals and humans that stem from its chemical behavior as a soft electrophilic α,β-unsaturated carbonyl compound. Evidence is presented that the nerve terminal is a primary site of ACR action and that inhibition of neurotransmission mediates the development of neurological deficits. At the mechanistic level, recent proteomic, neurochemical, and kinetic data are considered, which suggest that ACR inhibits neurotransmission by disrupting presynaptic nitric oxide (NO) signaling. Nerve-terminal damage likely mediates the neurological complications that accompany the occupational exposure of humans to ACR. In addition, the proposed molecular mechanism of synaptotoxicity has substantial implications for the pathogenesis of Alzheimer's disease and other neurodegenerative conditions that involve neuronal oxidative stress and the secondary endogenous generation of acrolein and other conjugated carbonyl chemicals.

KEYWORDS: Electrophile; toxic neuropathy; neurodegeneration; Alzheimer's disease; spinal cord injury; stroke

INTRODUCTION

Acrylamide (ACR) is used in a variety of industrial settings (e.g., water and wastewater management, cosmetic manufacturing, ore processing, and dye synthesis) and in scientific laboratories for the electrophoretic separation of macromolecules (*1–4*). In addition to occupational sources of exposure (**Table 1**), ACR is also present in cigarettes and is a significant food contaminant formed during the high-temperature preparation of certain potato- or grain-based products, for example, French fries, crackers, and bread (*5*). Although the precise chemistry has not been conclusively identified, it appears that the generation of ACR in certain food involves the formation of pyrolytic asparagine fragments, which is facilitated by the concomitant pyrolysis of Maillard-active dicarbonyl and hydroxycarbonyl precursors (*6, 7*). ACR is a well-documented neurotoxicant in both humans and laboratory animals. Subchronic, low-level occupational exposure of humans (**Table 1**) to ACR produces neurotoxicity characterized by ataxia, skeletal muscle weakness, and numbness of the hands and feet (*3, 8–10*). Daily exposure of laboratory animals to ACR is associated with progressive neurological signs that resemble the neurotoxicity occurring in humans, that is, ataxia and skeletal muscle weakness (*4, 11–13*). On the basis of a subchronic (90 day exposure) study of peripheral nerve damage in rats (*39*), a no observable adverse effect level (NOAEL) of 0.2 mg/kg/day has been established. Early morphological studies suggested that both human and experimental neurotoxicities were mediated by cerebellar Purkinje cell injury and by degeneration of distal axons in the PNS and CNS (*4, 14–17*). In addition to neurotoxicity, there is considerable experimental data from rodent studies that ACR produces reproductive toxicity, for example, reduced litter size, DNA strand breaks, and dominant lethal mutations (*18–20*). Furthermore, chronic (0.1−2.0 mg/kg/day × 2 years) rodent studies revealed an increased incidence of ACR-induced tumors in certain tissues, for example, mammary gland fibroadenomas in female rats and tunica vaginalis mesotheliomas in male rats (*21–24*). To date, however, epidemiological studies of occupationally exposed human cohorts have failed to establish a relationship between ACR exposure and an increased risk for cancer (*25–31*). Nonetheless, given the limited detectibility of subtle cancer risks inherent to human epidemiological research and significant evidence of carcinogenicity in experimental animals, ACR is considered to be a potential human carcinogen (IARC V.39, 1986; IARC S.7, 1987). Thus, ACR exposure is associated with neurotoxicity, carcinogenicity, and reproductive toxicity in laboratory species, whereas in humans, neurotoxicity is currently the only demonstrated effect of this toxicant.

Although carcinogenicity and reproductive toxicity are possible outcomes of exposure, ACR-induced neurotoxicity has documented and, consequently, immediate implications for human health. Moreover, our understanding of ACR neuropathy

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Table 1. ACR Exposure Parameters and Benchmark Dose Rates

average dietary intake (U.S.) = 0.4 μg/kg/day
contribution from smoking = 1.1−2.3 μg/cigarette
average occupational exposure level (worldwide) = 30 μg/kg/day
reference dose (RfD) = 0.20 μg/kg/day[a]
NOAEL[b] = 200 μg/kg/day
LOAEL[c] = 1000 μg/kg/day

[a] Established by the EPA in 1988. [b] No observed adverse effect level based on peripheral nerve damage in rats (*39*). [c] Lowest observed adverse effect level in rats (*39*).

and the underlying pathophysiological processes is relatively advanced due to more than 40 years of neuropathological characterization and abundant research investigating sites and mechanisms of action (*32–36*). This review will therefore discuss possible molecular pathophysiological processes that mediate ACR neurotoxicity. The focus will be on evidence that the nerve terminal is a primary site of ACR action and that inhibition of neurotransmission mediates the development of neurological deficits. At the mechanistic level, we will consider recent proteomic, neurochemical, and kinetic data, which suggest that ACR inhibits neurotransmission by disrupting presynaptic nitric oxide (NO) signaling. As will be discussed, the molecular mechanism of ACR-induced nerve-terminal damage has substantial implications for environmentally derived neurotoxicity and for the pathogenesis of Alzheimer's disease and other neurodegenerative conditions.

ACR-INDUCED NEUROLOGICAL DEFECTS AND NERVE-TERMINAL DAMAGE

The neurotoxicity of ACR has been extensively studied with respect to mammalian species including mice, rats, guinea pigs, cats, dogs, and monkeys at daily dose rates varying from 0.1 to 50 mg/kg/day (*32, 33, 38*). The overt signs of neurotoxicity are consistent across species, although the rodent appears to be the least sensitive species. In well-described rodent models, ACR intoxication at 5−50 mg/kg/day produces a triad of neurological deficits, that is, hind-limb foot splay, ataxia (open-field gait abnormalities), and skeletal muscle weakness such as decreased fore- and hind-limb grip strength (*11, 13, 39–43*). Experimental ACR intoxication is also associated with neurogenic autonomic dysfunction, for example, urinary retention, baroreceptor dysfunction, and impaired vasomotor control (*44–47*). The neurotoxicity of ACR is cumulative, and therefore neurological deficits develop progressively, at a rate determined by the daily dose; that is, higher ACR dose rates (30−50 mg/kg/day) produce severe neurotoxicity over a relatively short exposure duration (10−30 days), whereas lower dose rates (1−20 mg/kg/day) cause comparable neurotoxicity with significantly longer onset times [60 days−2 years (*11–13, 40–42*)]. A current point of ambiguity is whether this neurotoxicity is mediated by the parent chemical or by an active metabolite. Following exposure, ACR can be oxidized to an epoxide metabolite, glycidamide, presumably by the activity of cytochrome P450 2E1 (*48*). The results of one study (*49*) suggested that glycidamide played a causal role in producing the neurological deficits and axonal degeneration induced by ACR intoxication of rats. In contrast, other research has indicated that the parent compound (ACR) and not glycidamide is primarily responsible for induction of neurotoxicity (*50–54*). Additional research is clearly needed to define the role of glycidamide in the neurotoxic actions of ACR.

Early morphologic studies (*55–64*) revealed that low-dose subchronic induction of ACR neurotoxicity was associated with nerve damage in both the central and peripheral nervous systems (*33*). The morphological hallmark of this toxic neuropathy was considered to be distal preterminal axon swellings of the longest myelinated fibers. These swellings contained an abundance of neurofilaments, tubulovesicular profiles, and effete, probably degenerating, mitochondria (*59, 60, 64*). As exposure continued, progressive retrograde degeneration of these distal axon regions ensued with preservation of more proximal segments (*4*). On the basis of this pattern of neuropathological expression, Spencer and Schaumburg proposed that large-diameter axons in the CNS and PNS were most sensitive to the development of simultaneous, multifocal paranodal axon swellings in distal regions and that these swellings served as initiation points for subsequent degeneration. The characteristic spatiotemporal pattern of axon damage led to the classification of ACR neuropathy as a "central−peripheral distal axonopathy" (*65*). However, other morphological evidence generated during the past 30 years has indicated that early nerve-terminal damage might be importantly involved in the pathophysiological process leading to ACR neurotoxicity (*14, 59, 66–68*). Electrophysiological studies by Goldstein and Lowndes (*69–75*) showed that neurotransmission was impaired at spinal cord primary afferent nerve terminals as an early consequence of ACR intoxication of cats. Defective neurotransmission was also found at peripheral neuromuscular junctions and at autonomic synapses of ACR-intoxicated laboratory animals (*44, 68, 76*).

On the basis of evidence of early structural and functional damage, LoPachin and colleagues (*33*) suggested that nerve terminals were the primary site of ACR action and that synaptic dysfunction and subsequent degeneration were necessary and sufficient steps for production of ACR neurotoxicity. Corroborative research using the de Olmos silver stain method to detect neurodegeneration has shown that higher dose rate intoxication in rats (50 mg/kg/day) produced a selective terminalopathy characterized by very early, widespread nerve-terminal degeneration in gray matter regions of the spinal cord, brainstem, midbrain, and forebrain in the CNS (*77–79*). Intoxication of rats at a lower dose rate (21 mg/kg/day) caused initial nerve-terminal degeneration in PNS and CNS, which was followed by axon degeneration (*80*). Thus, regardless of dose rate, ACR caused initial presynaptic damage, which suggested that the nerve terminal was a primary site of action. In contrast, the dose-rate-dependent expression of axon degeneration indicated that this effect was not a significant neurotoxicological event (*33, 38, 81*). Nerve-terminal damage induced by ACR might be due to either a direct or an indirect effect. Thus, it is possible that deficient cell body synthesis and/or reduced delivery of presynaptic components causes secondary nerve-terminal damage (*32, 37, 82*). However, ACR does not affect perikaryal protein synthesis (*83–85*), and the morphological reorganization reported in nerve cell bodies (e.g., dorsal root ganglion neurons) of ACR-intoxicated animals appears to be a reparative reaction to axon/nerve-terminal damage and not a direct neuropathogenic effect (*86–89*). Although defective kinesin-based fast anterograde transport (FAT) has been studied as a mechanism of ACR-induced axon degeneration (*37*), other evidence indicates that such an effect does not play an important pathophysiological role (*51, 90–94*). Alternatively, nerve-terminal toxicity induced by ACR could develop secondary to an energy deficit (*35, 95*). However, it has been found that ACR exposure does not alter either anaerobic or aerobic energy production in central and peripheral nervous tissues (*96–99*). Together, these data suggest that, rather than being a secondary phenomenon, nerve-terminal damage is due to a direct effect

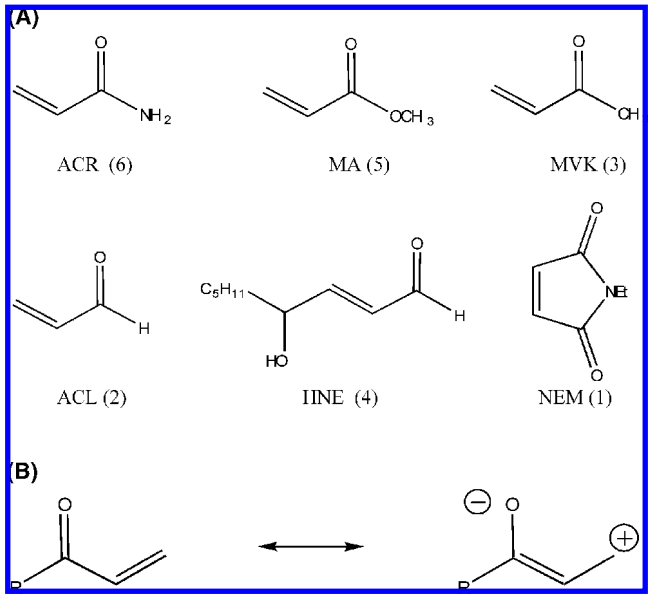

Figure 1. (**A**) Line structures for several conjugated α,β-unsaturated carbonyl derivatives of the type-2 alkene class. Numbers in paraentheses represent rank order of thiol reactivity (*101, 106*). ACR, acrylamide; MA, methyl acrylate; MVK, methylvinyl ketone; ACL, acrolein; HNE, 4-hydroxy-2-nonenal; NEM, *N*-ethylmaleimide. (**B**) Electron mobility (formation of a C3 carbocation) in the conjugated α,β-unsaturated carbonyl structure of a type-2 alkene.

of ACR. This possibility is supported by results from recent studies showing that direct, in vitro exposure of isolated brain synaptosomes to ACR decreased transmitter release (*100, 101*). The in vivo significance of this effect has been demonstrated by the observation that brain synaptosomes prepared from ACR-intoxicated rats were also release incompetent (*100*).

MOLECULAR MECHANISM OF ACR-INDUCED NERVE-TERMINAL DAMAGE

In developing a mechanistic theory for the presynaptic toxicity of ACR, we considered the well-documented adduct chemistry of this toxicant. ACR is an α,β-unsaturated carbonyl derivative and is, therefore, classified as a type-2 alkene (*101*). Many chemicals of this class are characterized by a conjugated system formed when the electron-withdrawing carbonyl group is linked to an alkene (**Figure 1A**). Due to the polarizable nature of the mobile π electrons (**Figure 1B**), these systems are able to undergo Michael-type conjugate additions with nucleophiles. According to the Molecular Orbital Theory, α,β-unsaturated carbonyl compounds are considered to be soft electrophiles that will react most favorably with soft nucleophiles (*102*) such as the sulfhydryl groups on cysteine residues in proteins (*103*). Such interactions in biological systems have been examined quantitatively (*104, 105*) through the use of quantum mechanical computations. In the case of ACR and the related compounds shown in **Figure 1A**, it has been shown that computed electronic properties (e.g., softness, electrophilic index, chemical potential) are well correlated to their kinetic constants for adduct formation and their toxicity in sulfhydryl-rich biological systems such as brain synaptosomes (*105, 106*). Because the function of many proteins that participate in neurotransmission and other critical processes is determined by the redox state of sulfhydryl groups on specific cysteine residues, we proposed that ACR alkylation of essential thiols impaired the activity of presynaptic proteins (*101, 105, 106*). Our research initially focused on *N*-ethylmaleimide (NEM) sensitive factor (NSF) as a target for ACR. Neurotransmitter release involves the fusion of synaptic vesicles with presynaptic membranes and the subsequent formation of a transmembrane pore (*103*). Membrane fusion is mediated by transient assembly of a soluble NSF attachment protein receptors (SNARE) core complex through the interactions of three presynaptic proteins: SNAP-25, syntaxin, and synaptobrevin. Disassembly of the fusion complex is the rate-limiting step of transmitter release and is mediated by the ATPase activity of NSF. As the name implies, NSF is inhibited by NEM and other sulfhydryl alkylating chemicals through adduction of Cys 264. This cysteine residue is located within domain I of the nucleotide-binding consensus sequence and is critically involved in ATP binding/hydrolysis (*107–109*). Our proteomic studies have demonstrated that ACR also formed adducts with this cysteine residue and that the resulting inhibition of NSF activity played a significant role in the reduction of neurotransmitter release that accompanied intoxication of rats (*110, 111*).

During subsequent investigations to establish neurotoxicological specificity of this NSF effect, it was found that ACR also inhibited other presynaptic processes, for example, neurotransmitter re-uptake and vesicular storage, by forming adducts with cysteine residues on respective proteins: the dopamine transporter, Cys 342, and v-ATPase, Cys 254 (*100, 111, 112*). This suggested that ACR influenced diverse nerve-terminal functions. Moreover, in vitro structure−toxicity studies demonstrated that other α,β-unsaturated carbonyls (e.g., acrolein, MVK, HNE) caused a similar broad-based presynaptic toxicity involving sulfhydryl adduction (*101*). These findings were consistent with previous studies, which showed that type-2 alkenes such as acrolein, acrylonitrile, and 4-hydroxy-2-nonenal (HNE) produced sulfhydryl-based neurotoxicity in vivo and in vitro (*113–118*). On the basis of these data, we proposed that ACR was a member of a large class of chemicals, the conjugated type-2 alkenes, that produced neurotoxicity via a common molecular mechanism involving cysteine adduct formation and nerve-terminal dysfunction (*101*). However, this hypothesis lacked molecular target specificity, because most proteins contain cysteine residues. This issue was addressed in a recent study, which showed that the highly nucleophilic sulfhydryl thiolate (RS^{-1}) state was the preferred type-2 alkene target (*105*). In biological systems at physiological pH (∼7.2), the thiolate concentration is low (∼10%) because the average pK_a of cysteine sulfhydryl groups is 8.5. Nonetheless, thiolates are more prevalent than predicted due to the existence of relatively low pK_a sulfhydryl groups on cysteine residues located within highly specialized amino acid sequences known as catalytic triads; for example, Cys 525 of glyceraldehyde-3-phosphate dehydrogenase is located within a catalytic triad and has a pK_a of 5.5. The thiolate state is formed by proton shuttling between flanking basic amino acid residues, such as histidine, arginine, or lysine, and their acidic counterparts aspartate and glutamate (*119, 120*). Catalytic triads are found within the active sites of many proteins (e.g., NSF, GAPDH, and vacuolar-ATPase) and play a critical role in modulating their function. Thus, cysteine thiolates of catalytic triads represent specific, mechanistically relevant targets for electrophilic neurotoxicants because these sulfhydryls are highly nucleophilic and are functionally critical. Our finding that ACR forms adducts with Cys 264 of NSF (see above) is, therefore, significant because this residue is located within the catalytic triad of the nucleotide-binding site of domain I and is responsible for modulating the ATPase activity of this enzyme. Furthermore, type-2 alkene adduction of Cys 264 inhibits NSF activity, which secondarily disrupts the synaptic vesicle cycle

and other presynaptic membrane fusion processes (*107–111*). In contrast, data from several in vitro studies have suggested that neurotoxicity may be mediated by cellular oxidative stress following type-2 alkene depletion of glutathione (*121–123*). However, it has recently been shown (*112*) that ACR intoxication does not affect CNS neuronal redox state in whole animal models (see also, ref *124*). Furthermore, numerous studies have indicated that the formation of protein adducts (and not GSH depletion) is the primary pathogenic step in type-2 alkene toxicity (*125–129*). These data strongly suggest that ACR, and other type-2 alkenes, produce neurotoxicity by forming adducts with sulfhydryl thiolates in cysteine catalytic triads.

The cysteine thiolate residues of catalytic triads are acceptors for nitric oxide (NO) signaling and, predictably, most of the protein targets of ACR (e.g., NSF, DA transporter, and v-ATPase) are also recognized effectors for NO signaling (*101, 110, 111*). Therefore, it seems likely that the type-2 alkenes produce neurotoxicity by interfering with this pathway. NO is a biological electrophile that reversibly adducts thiolates (S-nitrosylation) and thereby regulates the activities of many nerve-terminal proteins and their respective pathways (*120, 130*). It was originally thought that NO influenced cellular processes through stimulation of soluble guanylyl cyclase (*131*). However, more recent research indicates that NO rapidly modulates many processes by forming reversible adducts with thiolates (S-nitrosylation) of catalytic triads. S-Nitrosylation, therefore, represents a redox-based signaling mechanism that regulates neurotransmission in a fashion similar to the posttranslational modifications induced by protein phosphorylation (*132*). The specificity and functional independence of NO-mediated actions is a product of signaling modules that act as neuronal microprocessors. For example, stimulus-evoked generation of NO via the neuronal synthetic enzyme, nNOS, occurs in proximity to the effector element, the thiolate of a corresponding catalytic triad. The resulting oxidation of the thiolate by NO specifically modulates protein function (e.g., the NMDA receptor complex; see ref *133*). Although controversy exists regarding the effects of NO signaling at the nerve terminal, the weight of evidence suggests a reduction in synaptic strength through binary ("on−off") regulation of many presynaptic processes; that is, NO inhibits synaptic vesicle membrane fusion and decreases both membrane neurotransmitter uptake and vesicular storage (*134*). Our studies suggest that ACR and the type-2 alkenes mimic these neurophysiological effects of NO signaling (*100, 101, 112*). Therefore, oxidation of thiolate anionic sites in protein catalytic triads by either reversible S-nitrosylation (endogenous NO) or irreversible alkylation (exogenous ACR) produces similar synaptic effects that differ with respect to duration of effect and outcome—neuromodulation versus neurotoxicity, respectively. Because ACR and other type-2 alkenes form irreversible adducts with NO-targeted thiolates, we hypothesize that NO signaling is blocked and that the loss of reversible, spatially precise neuromodulation produces nerve-terminal toxicity.

SELECTIVE VULNERABILITY OF NERVE TERMINALS TO ELECTROPHILIC ATTACK

NO modulation of protein catalytic triads is a generic cellular, which seems incompatible with a proposed mechanism of selective nerve-terminal damage. However, several unique anatomical and functional characteristics predispose this neuronal region to electrophilic attack (reviewed in refs *103* and *106*). Thus, it is notable that presynaptic function is regulated extensively by NO signaling. Electrophile-induced disruption of NO modulation could, therefore, have broad functional consequences for neurotransmission. Because the nerve terminal is anatomically separated from the cell body, it is devoid of transcriptional or translational capacity. As a consequence, it lacks the ability to initiate transcriptionally based reparative and protective responses; for example, the nerve terminal cannot mount a protective response to an electrophile mediated by the NEPP-Keap1/Nrf2 pathway. Furthermore, because nerve terminals cannot manufacture proteins, cell body synthesis and subsequent anterograde axonal transport maintain the presynaptic proteome. As a conservation mechanism, the turnover of nerve-terminal proteins is slow relative to that of proteins in other nerve regions or cell types (*103, 106, 111*). Consequently, when such proteins are adducted and rendered dysfunctional, they are replaced slowly. With continued toxicant exposure, the dysfunctional protein pool grows and the related cellular pathways are progressively disabled. Given this proteome toxicodynamic, cumulative toxicity with parallel accumulation of protein adducts in target cells is the predicted outcome. Experimentally, both cumulative neurotoxicity and consonant adduct accumulation have been demonstrated (*13, 110, 111*). In contrast, adducted, dysfunctional proteins with short half-lives will not accumulate (i.e., they are rapidly replaced) and, consequently, will have minimal toxic impact.

TOXICOLOGICAL CONSEQUENCES OF TYPE-2 ALKENE ENVIRONMENTAL EXPOUSRE

As stated above, ACR is a member of a large class of structurally similar toxic chemicals, the type-2 alkenes (**Figure 1A**). These chemicals are soft electrophiles that produce toxicity through a common molecular mechanism involving the formation of adducts with soft nucleophilic cysteine residues. Conjugated α,β-unsaturated carbonyl and acrylic acid type-2 alkenes have extensive commercial and industrial applications, and they are well-documented environmental pollutants and food contaminants (*1, 135–144*). Consequently, humans are pervasively exposed to chemicals in this class. However, although neurotoxicity is a clearly defined outcome in ACR-exposed human cohorts (*3, 4, 55*), systemic exposure to acrolein, MVK, or other type-2 alkenes is primarily associated with respiratory, hepatic, or renal toxicity (**Table 2**) (*136, 145–147*). This toxicological diversity is not related to differences in molecular mechanisms among these chemicals (*101, 105, 139, 140, 148*), but is instead due to relative differences in electrophilic reactivity and the resulting impact on tissue distribution (*149–152*). Acrolein, NEM, and MVK are highly electrophilic and, therefore, rapidly form adducts with sulfhydryl thiolate groups. Following systemic intoxication with reactive type-2 alkenes, the rapid formation of protein adducts essentially limits tissue distribution (*153*) and, as a consequence, the resulting toxic manifestations are determined by the site of absorption; for example, inhalation of acrolein produces pulmonary toxicity, whereas systemic administration is associated with hepatic and vascular toxicity (*135, 154–156*). In contrast, ACR is a weak water-soluble electrophile that forms thiolate adducts slowly. It is, therefore, less susceptible to the limiting influence of systemic "adduct buffering" and has a correspondingly larger volume of distribution that encompasses the CNS (*50, 157–159*). Thus, although it might seem counterintuitive, the greater threat of environmentally derived neurotoxicity comes from exposure to weaker electrophiles (e.g., ACR, methyl acrylate), whereas environmental exposure to softer, more reactive, electrophiles (e.g., acrolein) is likely to produce systemic toxicity (i.e., hepatotoxicity).

Table 2

type-2 alkene[a]	source[b]	toxicity[c]	electrophilicity (ω)[d]
NEM	O	systemic	4.73
ACL	O, E, endog	systemic/NTX	3.57
HNE	endog	NTX	3.29
MVK	O, E	systemic	3.00
MA	O, E, D	NTX	2.76
ACR	O, D	NTX	2.30

[a] NEM, *N*-ethylmaleimide; ACL, acrolein; HNE, 4-hydroxy-2-nonenal; MVK, methylvinyl ketone; ACR, acrylamide; MA, methyl acrylate. [b] O, occupational (e.g., chemical industry, agriculture, manufacturing); E, environmental (e.g., cigarette smoke, combustion of organic material, auto exhaust); endog, endogenous cellular production secondary to oxidative stress; D, dietary. [c] Systemic, renal, hepatic, or pulmonary toxicity depending upon route; NTX, neurotoxicity from either exogenous (e.g., environmental) or endogenous (e.g., following lipid peroxidation) exposure. [d] To calculate electrophilicity (ω), the lowest unoccupied molecular orbital energy (E_{LUMO}) and the highest occupied molecular orbital energy (E_{HOMO}) were calculated using Spartan04 (version 1.0.3) software (Wave Function Inc., Irvine CA). Single-point energies for each structure were calculated at the density functional (DF) level of theory using a B3LYP-6-31G* basis set from 6 to 31G* geometries. Electrophilicity was calculated as $\omega = \mu^2/2\eta$, where global (whole molecule) hardness (η) was calculated as $\eta = (E_{LUMO} - E_{HOMO})/2$ and chemical potential (μ) was calculated as $\mu = (E_{LUMO} + E_{HOMO})/2$ (*105*). Electrophilicity is an index of electrophile reactivity; that is, larger values denote more electrophilic reactivity.

RELEVANCE OF ENDOGENOUS TYPE-2 ALKENE PRODUCTION TO HUMAN DISEASES

In addition to the toxicity of environmental exposure, a large database suggests that endogenous generation of type-2 alkenes plays a major pathogenic role in acute neurotrauma (e.g., spinal cord trauma) and certain chronic neurodegenerative diseases (e.g., Alzheimer's disease). The neuronal injuries associated with these conditions share a common pathophysiological cascade that involves oxidative stress and lipid peroxidation (*160–162*). The free radical driven peroxidation of polyunsaturated fatty acids damages cellular membranes and generates neurotoxic α,β-unsaturated carbonyl derivatives such as acrolein and HNE (*162–164*). Accordingly, evidence from animal models and human cohorts suggests that liberation of these reactive α,β-unsaturated aldehydes and subsequent formation of protein adducts mediate the neuronal injury induced by stroke or acute spinal cord trauma (*165–168*). A relatively large database suggests that Alzheimer's disease (AD) is characterized by an oxidative cascade and damage to nerve terminals (*162, 169–173*). In AD, presynaptic toxicity antedates frank neurodegeneration and is, therefore, considered to be a primary component of the neuropathogenic process (*169, 171, 174, 175*). The mechanism of AD synaptotoxicity has not been elucidated (*174–178*), although numerous studies have reported elevated levels of HNE, acrolein, and their respective protein adducts in relevant brain regions (e.g., amygdala and hippocampus) of AD patients and transgenic animal models (*170, 179–186*). Our research suggests a causal relationship between presynaptic dysfunction and the liberation of type-2 alkenes in the AD brain; that is, acrolein and HNE form irreversible adducts with NO thiolate acceptors on presynaptic proteins, and the resulting loss of NO regulation causes synaptic dysfunction and eventual degeneration. Thus, the memory and cognitive deficits of AD might develop as a consequence of regional presynaptic type-2 alkene toxicity. Furthermore, because type-2 alkenes share a common mechanism of presynaptic toxicity, it is possible that environmental exposure to weak electrophilic members of this chemical class (e.g., ACR in food or occupational exposure to MA) accelerates the nerve-terminal damage induced by endogenous acrolein/HNE. Finally, the pathophysiological relevance of acrolein and HNE extends well beyond neurotrauma and neurodegeneration, because these type-2 alkenes appear to be importantly involved in the pathogenesis of many systemic diseases also associated with lipid peroxidation, for example, diabetes, atherosclerosis, pulmonary diseases, and inflammatory conditions (*135, 187*).

SUMMARY

ACR is a type-2 alkene and has demonstrable neurotoxic effects in both laboratory animals and humans that stem from its chemical behavior as a soft electrophilic α,β-unsaturated carbonyl compound. Other type-2 alkenes (**Figure 1A**) react in biochemical systems via a common mechanism but might or might not be neurotoxic depending upon the extent of their ability to behave as electrophiles. Evidence suggests that the primary site of ACR action is the nerve terminal and that inhibition of neurotransmission mediates the development of neurological deficits. At the mechanistic level, recent proteomic, neurochemical, and kinetic data suggest that ACR inhibits neurotransmission by disrupting presynaptic NO signaling. The proposed molecular mechanism of ACR-induced nerve-terminal damage has substantial implications for environmentally derived neurotoxicity and for the pathogenesis of Alzheimer's disease and other neurodegenerative conditions, because the resulting loss of NO regulation causes synaptic dysfunction and eventual degeneration.

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