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CONTEMPORARY ISSUES IN TOXICOLOGY

Excitotoxins, Aging, and Environmental Neurotoxins: Implications for Understanding Human Neurodegenerative Diseases

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We are an aging society and current demographic trends point to a likely increase in age-related neurodegenerative diseases. The aged population may have a number of unique risk factors that result in a predisposition to neuronal damage from environmental neurotoxins. This symposium addressed the involvement of excitatory amino acids as final common mediators of neuronal death associated with various types of neurotoxic insult. The roles of oxidative stress, mitochondrial energy metabolism, and disruption of calcium homeostasis were discussed in relation to excitotoxicity and several experimental models of human neurodegenerative diseases. The neurotoxic actions of kainic acid, 3-nitropropionic acid, cyanide, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, and methamphetamine were examined for their relevance as models of human neurodegenerative disorders. The mechanisms of action of excitotoxins in experimental models of Huntington's disease and Parkinson's disease were explored in light of the enhanced susceptibility and potential vulnerability of the aged nervous system to neurotoxins that perturb cellular metabolism and homeostatic processes. Bioenergetic defects and oxidative stress were found to be critical links in a neurotoxic cascade of events that trigger the sustained release of excitotoxic amino acids. The interrelationships among the aging process, the pathophysiology of neurodegenerative diseases, and the mechanism of action of various neurotoxins were addressed from the unifying perspective of the excitotoxic hypothesis of neuronal death. © 1995 Academic Press, Inc.

We are an aging society in both absolute numbers and proportionately. Dementia and neurodegenerative diseases in the aging population are major and growing medical and social problems. Dementia has its highest incidence in the population over age 75, which is the fastest growing age

group. By 2020, 16% of the total population is projected to be elderly. Reports by the Congressional Office of Technology Assessment (1990) and the National Research Council (1992) have both concluded that environmental and industrial neurotoxicants pose a risk to the general population and highlighted the elderly as a special "at risk" population. Research in the area of neurotoxicology needs additional focus in exploring the special risk factors to neurotoxic insult exhibited by our elderly population. Research in the fields of aging and neurodegenerative diseases is in the forefront of basic and clinical neuroscience. The special expertise of the toxicologist needs to be focused on the toxicokinetic and pharmacodynamic actions of chemicals, drugs, and environmental agents and the problems they pose to the elderly population. This symposium focused on the role of excitatory amino acids as common mediators of neuronal cell death in neurological disorders and the aging CNS. The unique susceptibility and vulnerability of the aged CNS to direct- or indirect-acting excitotoxins were highlighted by the speakers and the model systems that they use to study these problems.

The term "excitotoxin" was coined by John Olney in the 1970s to describe the neurotoxic properties of the acidic amino acids, glutamate (GLU) and aspartate (ASP), and their analogues (Olney, 1980). These endogenous amino acids were known since the early 1960s to produce membrane depolarization of neurons when ionophoretically applied (Curtis *et al.*, 1960). Since then, other endogenous substances have been found to be potential excitatory neurotransmitters (Table 1). The neurotoxic actions of GLU on the retina were first recognized by Lucas and Newhouse (1957), and Olney (1980) conducted his pioneering characterization of excitotoxic amino acids in the CNS in the 1970s. Currently, the study of the pharmacology, neurochemistry, and neurotoxicology of excitatory amino acids is one of the most active areas in neuroscience, including

TABLE 1
Some Endogenous and Exogenous Excitotoxins

Endogenous excitotoxins	Exogenous excitotoxins
Glutamate	Kainic acid
Aspartate	Domoic acid
Quinolate	β -N-Oxalamino-L-alanine (BOAA)
N-Acetyl-aspartylglutamate (NAAG)	β -N-Methylamino-L-alanine (BMAA)
L-Homocysteate	Ibotenic acid
L-Cysteine sulfinate	Quisqualic acid
L-Cysteine	Willardiine

the study of their clinical significance in neurological disorders (Choi, 1988; Lipton and Rosenberg, 1994; Table 2). GLU and ASP are considered to be the predominant excitatory neurotransmitters in the CNS, although other candidate neurotransmitters exist (Table 1). There are a number of naturally occurring excitotoxic amino acids, which include kainic acid, domoic acid, and BMAA (Table 1). Excitatory amino acids (EAAs) are involved in learning and memory, sensory processing, cardiovascular and neuroendocrine control, the motor system, and numerous other vital functions (Daw *et al.*, 1993, van den Pol and Trombley, 1993). The anatomy of the major pathways, which utilize EAA as neurotransmitters, is well known (Storm-Mathisen and Ottersen, 1988) and there has been an explosive growth in the knowledge of the receptor pharmacology of EAAs based on molecular cloning studies of receptor subtypes and subunits (Nakanishi, 1992, Gasic and Hollmann, 1992). Four receptor subtypes have been extensively characterized by both molecular and functional studies (Table 3). Other subtypes exist and are currently being more fully

TABLE 2
Neurodegenerative Diseases and Neurological Disorders Linked to Abnormal Excitatory Amino Acid Metabolism

Olivopontocerebellar atrophy
Amyotrophic lateral sclerosis
Huntington's disease
Alzheimer's disease
Parkinson's disease
Temporal lobe epilepsy
AIDS-related dementia
Stroke/ischemia

TABLE 3
The Major Classes of Glutamate Receptor Subtypes

Subtypes	Effector systems
NMDA ^a	Cation channel (Ca ²⁺ , Na ⁺ , K ⁺)
AMPA ^b	Cation channel (Na ⁺ , K ⁺)
Kainate (KA)	Cation channel (Na ⁺ , K ⁺)
Metabotropic	G proteins (G _i , G _o)

^a N-Methyl-D-aspartic acid.

^b Amino-3-hydroxy-5-methylisoxazole-4 propionic acid.

characterized. Excitotoxins have been powerful tools with great experimental and heuristic value to neurotoxicologists and neuroscientists.

A key purpose of this symposium was to provide information and stimulate discussion about the links between neurotoxicants, the aging processes, and human neurodegenerative diseases. This introduction will be a starting point for the discussion of some of these potential links and will also briefly describe the factors that contribute to turning excitatory amino acids from neurotransmitters to neurotoxins. GLU is unique among other neurotransmitters in requiring a relationship with glia for both normal biosynthesis and inactivation (Fig. 1). GLU is a two-edged sword as a neurotransmitter since "too little or too much" results in functional and/or pathologic consequences.

Some interactions that merit further study in regard to toxic agents, aging, and excitotoxicity are listed in Table 4. These processes relate to both the pre- and the postsynaptic events involving GLU efflux and inactivation, receptor

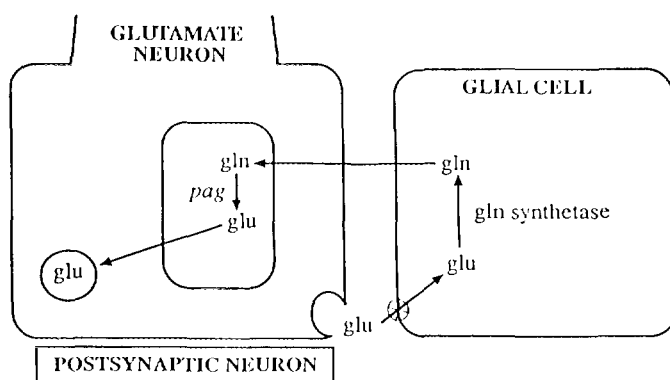


FIG. 1. Overview of the neuronal-glial glutamate cycle. GLU is synthesized from GLN by the mitochondrial enzyme, PAG. GLU is stored in synaptic vesicles and is also present in high concentrations of the cytoplasm. GLU is released and taken up by both glia and neurons by a high-affinity, sodium-dependent transport system. Glial cells convert GLU to GLN via the enzyme glutamine synthetase and GLN can be taken up by neurons.

TABLE 4
Potential Links between the Aging Process, Excitotoxins,
and Environmental Neurotoxicants

Energy metabolism
Antioxidant capacity
Protein modification
Calcium homeostasis
Ammonia detoxification
Growth factor regulation

stimulation, and postreceptor events. The exact processes that lead to neuronal death are multifaceted and complex. A trigger event must occur that results in a loss of control of the compartmentation of EAAs. GLU efflux can be calcium-dependent or -independent and involves release from synaptic vesicles or direct cytosolic release (Nicholls, 1993; Bernath, 1991). There is an approximately 10,000-fold difference between the 10 mM intracellular concentration of GLU and the $\approx 1 \mu\text{M}$ concentration normally found in the brain extracellular space. The dissipation of the normal ionic gradients due to excessive depolarization or energy collapse results in the massive release of nonneurotransmitter GLU and this has been shown to be the mechanism of both ischemic and hypoxia neuronal death (Choi, 1992). A number of postreceptor events occur that result in the amplification and expression of neuronal death (Choi, 1992). These involve ionic events, energy depletion, calcium-mediated enzymatic events, and free radical generation. These mechanisms are further explored and elaborated on in the following sections.

A substantial body of research has focused on the role of GLU in aging and neurological diseases. The release mechanisms and biosynthesis of GLU will be briefly reviewed as a starting point for the discussion of the role of GLU in neurodegenerative processes and neuronal death. Phosphate-activated glutaminase (PAG) is the major enzymatic pathway in forebrain structures for neurotransmitter biosynthesis (Erecinska and Silver, 1990), whereas in the brainstem ASP aminotransferase is important (Kihara and Kubo, 1989). PAG generates both GLU and NH_4 , which are products that are potentially toxic (Fig. 1), and both act as powerful end product inhibitors of the enzyme. The aging nervous system has been found to be significantly less responsive to ammonia feedback inhibition of PAG (Wallace and Dawson, 1992). This age-related deficit is specific to certain brain regions and end product inhibition by GLU and GLU analogues appears intact (Dawson and Wallace, 1993). Calcium activation and phosphate activation of PAG are also diminished in the aged nervous system (Wallace and Dawson, 1993). In general, ammonia levels in-

crease in the brain as a function of age, whereas GLU content tends to decrease in cortical areas (Rajeswari and Radha, 1984; Wallace and Dawson, 1990). Recently, Seiler (1993) has proposed that abnormal ammonia detoxification may contribute to the pathophysiology of neurodegenerative diseases. Data from the laboratory of Dawson and co-workers would support an age-related deficit in ammonia handling, which would also alter GLU synthesis and release. Calcium-dependent GLU release appears normal or elevated in aged rats depending on the brain region and intensity of depolarization conditions (Dawson *et al.*, 1989; Meldrum *et al.*, 1992a; Cobo *et al.*, 1993; Palmer *et al.*, 1994). If the aged brain is less responsive to feedback inhibition from NH_4 as shown by Wallace and Dawson (1992), then agents that perturb release may be more effective in sustaining prolonged periods of excessive GLU release or delayed GLU inactivation. A number of neurotoxicants are known to influence GLU (Table 5). By contrast, metabolic extremes like hypoxia and ischemia produce severe but acute elevations of GLU efflux and neuronal death (Benveniste *et al.*, 1989). Neurodegenerative processes are more insidious in nature and are more likely related to modest but sustained elevation of extracellular EAA concentrations. Candidate mechanisms for neurotoxic agents acting as indirect excitotoxins would include altered synthesis, release, or reuptake of GLU or the stimulation of nonspecific efflux of GLU from the cytosolic compartment of neurons or glia. Trimethyltin (TMT) produces substantial damage to the hippocampus and related limbic and cortical areas (Brown *et al.*, 1979). TMT has been shown to stimulate

TABLE 5
Some Environmental Neurotoxins and Their Effects
on Glutamate

Toxin	Effects on glutamate
Trimethyltin	Increased glutamate release Glutamate uptake blockade
Ethanol	Decreased glutamate release Blockade of NMDA receptor function
Aluminum	Decreased glutamate release
Methylmercury	Increased glutamate release
Cyanide	Increased glutamate release
Manganese	Inhibition of uncoupled mitochondrial respiration with glutamate as substrate
Toluene	Increases in glutamate receptor binding
Lead	Decreased glutamate content in the motor cortex
Permethrin	Increased glutamate content in the brainstem
Diazinon	Decreased cortical content of ASP and GLU

GLU efflux from brain (Naalsund and Fonnum, 1986); however, TMT-stimulated GLU release does not differ between adult and aged F344 rats (Dawson *et al.*, 1995). Differences may also exist in the responsiveness of the aged nervous system to excitotoxins based on age-related alterations in receptor binding or coupling. In general, *N*-methyl-D-aspartate (NMDA) receptor number decreases in the aged nervous system (Tamaru *et al.*, 1991; Wenk *et al.*, 1991; Magnusson and Cotman, 1993), but it is unknown whether this is due to a loss of vulnerable neurons with NMDA receptors or an adaptive downregulation to protect aged neurons. It was reported that elderly individuals that consumed domoic acid-contaminated mussels had more severe neurological deficits than younger exposed individuals (Teitelbaum *et al.*, 1990; Auer, 1991). Domoic acid acts at the kainic acid (KA) receptor subtype and KA receptor number does not decrease with age (Tamaru *et al.*, 1991). Dawson and Wallace (1992) examined the sensitivity of aged female rats to kainic acid-induced seizures and found aged animals to be approximately fourfold more sensitive than adult rats. These aged animals also showed a greater fall in brain GLU and ASP content 2 hr after kainic acid treatment (Dawson and Wallace, 1992). Dawson and Wallace (1992) also found KA to induce a significantly greater increase in ASP release from brain slices of aged rats. These studies suggest that aged animals are compromised in response to excessive activation of KA receptors. Olney and co-workers (1991) have reported similar findings and these data tend to substantiate the human data on domoic acid toxicity.

The aged nervous system is also known to have compromised brain glucose utilization and energy metabolism (Hoyer, 1990). It is also known that conditions that compromise neuronal energy metabolism greatly exacerbate the toxicity of exogenous EAA (Novelli *et al.*, 1988). Hypoglycemia is known to increase the stimulated release of ASP and GLU (Burke and Nadler, 1989). The effects of hypoglycemia on potassium-stimulated GLU and ASP release in aged rats have previously been examined and found to be augmented to the same level as that of adult controls (Meldrum *et al.*, 1992b). Subsequent authors will also address the pivotal role of energy metabolism and mitochondrial function in age-related neurodegenerative processes.

The ability of GLU receptor activation to induce calcium-dependent processes related to free radical production and the resulting protein and lipid modifications that compromise mitochondrial and cell membrane function all interrelate to potential mechanisms for sustained and progressive neuronal damage and compromise. These phenomena are important given the research literature that points to the aged nervous system having diminished antioxidant defense mechanisms (Bondy and LeBel, 1993), deficient neuronal glucose and energy metabolism (Hoyer,

1990), limited functional reserve capacity, aberrant calcium homeostasis (Gibson and Peterson, 1987; Landfield *et al.*, 1992), and reduced protein repair mechanisms (Stadtman, 1988). Neurotoxins that inhibit electron transport in mitochondria or inhibit the production of high energy phosphate compounds lead to energy failure and both enhanced GLU release and increased vulnerability to extracellular GLU (Lipton and Rosenberg, 1994). Furthermore, GLU activation of NMDA receptors enhances nitric oxide formation which can contribute to free radical-mediated neurotoxicity (Lipton and Rosenberg, 1994). Free radical-mediated oxidative damage can result in a loss of glutamine synthetase activity (Oliver *et al.*, 1990) and can also lead to increased levels of brain GLU. Neurotoxicants with diverse specific mechanisms of action may ultimately trigger an excitotoxic cascade in the aged nervous system. The subsequent sections will explore the links among mitochondrial function, oxidative stress, and EAAs.

THE RELATION BETWEEN EXCITOTOXICITY AND OXIDATIVE STRESS

This outline is not intended to constitute a thorough review but is intended to illustrate that a complex relation exists between excitatory and oxidative events in the nervous system. The modeling of neurological diseases using selected toxic chemicals described in this article reveals that interactions between these two modalities are likely to play a role in both age-related and environmentally caused changes in nerve function. This subject is described in greater detail in a recent review (Bondy, 1995). All of the other sections constituting the current overview also describe agents that involve both oxidant and excitatory events acting in concert. Thus, such a "double hazard" combination is suspected to account for the neurotoxicity of glutamate, 3-nitropropionic acid, cyanide, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

Many toxic chemicals disrupt the production of cellular energy reserves and therefore impair anabolic processes. This may be by inhibition of mitochondrial oxidative phosphorylation or by reduction of the supply of adequate nutrients to the cell. Another large class of toxic chemicals causes an excess energy demand by interfering with the stabilization of energy-requiring events such as maintenance of ionic gradients, for example, by compromising membrane integrity. Thus, one of the most common events that may occur as a result of exposure to a toxicant is excess demand on, or deficient supply of, the energy requirements of the cell. A broad range of unrelated chemicals may have in common the ability to disrupt the balance between cellular ATP supply and demand. This general feature may lead to the emergence of pathological changes that reflect the susceptibilities of the cell rather than being related more to a specific feature of the toxic agent.

In the case of the central nervous system, several distinctive biochemical and anatomical features lead to commonly recurring characteristics of expression of neurotoxicity. These features may be considered "weak points" in that they are the first to become deranged both by neurotoxic agents and in neurological disorders. One of these vulnerable features involves the uniquely fluctuating demand made by nervous tissue upon intracellular concentrations of cations. Maintenance of cationic gradients is required by all tissues. Yet in order to allow nervous tissue function, these gradients must be allowed to dissipate and then to be rapidly restored. These fluxes are needed for the passage of action potentials, the modulation of postsynaptic potentials, and the calcium-effected release of neurotransmitters. This continuously changing ion gating causes entropy-raising reductions in the steepness of ionic gradients. The gradients need to be rapidly restored by entropy-requiring ion pumping or sequestering events. This demand largely accounts for the high energy requirement of the CNS.

Failure to restore homeostasis after a depolarizing event very commonly leads to neuronal hyperexcitability. This is due both to the attenuation of the axonal resting potential due to excess intracellular sodium and to increased levels of cytosolic calcium leading to abnormal presynaptic neurotransmitter release. In the absence of adequate supplies of energy, both of these events may be increasingly accelerated. The collapse of ion gradients may account for the appearance of hyperactivity, tremor, and convulsions that commonly accompany many neurotoxic and neurological insults. While each deleterious chemical has its own specific means of cellular disruption, the commonality of hyperexcitation is superimposed on any distinctive features.

Another recurring characteristic weak point of the suboptimally functioning cell is its tendency to generate excessively high levels of reactive oxygen species (ROS) (free radicals). ROS may be oxygen-centered radicals possessing unpaired electrons, such as superoxide anion and hydroxyl radical, or covalent molecules such as hydrogen peroxide (Halliwell and Gutteridge, 1989). Disruption of the electron transport chain can lead to the appearance of incompletely reduced oxygen species. Some of these moieties, such as the hydroxyl radical, have extremely short half-lives and they rapidly oxidize any organic molecule in their proximity. Appearance of excessive levels of ROS is generally symptomatic of inefficient mitochondrial combustion of substrates.

Antioxidant defense systems can be overwhelmed by elevated levels of ROS production. Mechanisms include induction of mixed function oxidases or conversion of xanthine dehydrogenase to xanthine oxidase. Oxidases undertake an inherently hazardous task involving scission of the relatively inert oxygen molecule. This invariably involves the appearance of an active oxidizing species. How-

ever, under normal conditions, appropriate protective systems minimize the intensity of prooxidant activity. For example, mitochondrial cytochrome oxidase has superoxide dismutase activity and the rate of leakage of free radicals out of healthy mitochondria is very low. Another means by which exposure to a toxicant can lead to abnormal rates of production of ROS is by excessive utilization of glutathione in detoxifying conjugation or reduction reactions.

A variety of changes resulting from an impaired balance between the supply and the demand of intracellular energy can also lead to an imbalance between the rates of production and inactivation of ROS. Excess demand for ATP production or reduced availability of oxygen, as in ischemia, can compromise mitochondrial efficiency and lead to the appearance of excess ROS.

Both hyperexcitability and excess ROS generation are relatively nonspecific consequences of neural insult and may be associated with an extensive and varied range of neurotoxic exposures. There is clearly a relation between these two symptoms of cellular malfunction. It has been proposed that during ischemia, ROS and excitatory amino acids may cooperate in effecting neuronal damage (Bose *et al.*, 1992). However, the two phenomena are distinct entities and, while they are often incurred simultaneously, the causal relation between them is not always clear. There is evidence of a reciprocal activation of these processes. Thus, the influx of and prolonged elevation of calcium that may occur under conditions of energy deficiency may activate ROS generation by:

(i) Activation of the arachidonic acid cascade. The enzymic conversion of this compound to prostaglandins, leukotrienes, and thromboxanes by cyclooxygenases and lipoxygenases which directly utilize molecular oxygen leads to considerable ROS generation and this may be a means by which excitatory events promote excess generation of ROS (Pellerin and Wolfe, 1991).

(ii) Stimulation of lipolysis and, thence, lipid peroxidation (Vanella *et al.*, 1992).

(iii) Depression of oxidative phosphorylation and derangement of mitochondrial function (Zhang *et al.*, 1990).

(iv) Catalysis of conversion of xanthine dehydrogenase to xanthine oxidase (McCord, 1987).

Conversely, there is also evidence that:

(i) ROS can mobilize intramitochondrial stores of sequestered calcium (Richter and Kass, 1991).

(ii) ROS inhibits calmodulin-dependent processes, thereby elevating levels of cytosolic free calcium (Okabe *et al.*, 1989).

(iii) ROS can lead to extracellular release of excitatory amino acids (Pellegrino-Giampeiro *et al.*, 1990).

The therapeutic possibilities resulting from recognition

of these two overlapping "final common pathways" of neurological insult include the use of antioxidant and calcium channel-blocking drugs. These approaches are finding increasing utility in the treatment of many neurological disorders, including Parkinson's disease, Alzheimer's, multiple sclerosis, epilepsy, amyotrophic lateral sclerosis, stroke, and cerebral ischemia (Bondy and LeBel, 1993). Several common neurological diseases are suspected to involve a combination of interacting excitatory and oxidative processes (Halliwell, 1989; Coyle and Puttfarcken, 1993; Lipton and Rosenberg, 1994). More prolonged treatments emphasize the use of antioxidant vitamins, while calcium channel blockers and iron chelators find application in more acute situations or in intermittent therapies. The maintenance of low levels of iron and calcium within the cell is essential to the establishment of an optimal milieu for anabolic biochemical processes. A small increase in levels of free iron within cells can dramatically accelerate rates of ROS production (Minnotti and Aust, 1989), and calcium channel blockers can prevent cyanide-induced lipid peroxidation and cell death (Maduh *et al.*, 1988).

While the treatment of a neurotoxic exposure obviously needs to include procedures targeted toward mitigation of the specific properties of the toxicant, the addition of more general therapies, focused on protection against these recurrent vulnerabilities of the CNS, is also of importance. This approach is warranted in excitotoxin-mediated neuronal damage due to the prominent roles of calcium and ROS in the mechanisms of cell death.

EXCITOTOXICITY IN HUNTINGTON'S DISEASE

The concept that excitotoxicity may play a role in the pathogenesis of Huntington's disease (HD) was suggested in 1976 after it was shown that kainic acid lesions in the striatum of rats could reproduce many of the characteristic features of HD (reviewed in Beal, 1992). Beal and co-workers later showed that NMDA agonists such as quinolinic acid provide an improved model since they spare striatal interneurons, such as NADPH-diaphorase neurons, which are spared in HD. However, it is difficult to envision how an acute excitotoxic process could occur in HD, which evolves over 10–15 years. One possibility is that a low-grade disturbance of energy metabolism may lead to slow excitotoxic neuronal degeneration in HD (Beal, 1992). An impairment of energy metabolism may result in neuronal depolarization, which can then lead to activation of voltage-dependent NMDA receptors. This leads to an influx of calcium and cell death by excitotoxic mechanisms. One of the downstream mechanisms is the generation of free radicals. The generation of free radicals by mitochondria is markedly augmented by increases in intracellular calcium in the range of those achieved following activation of NMDA receptors.

If this theory of cell death is valid then one should be able to produce selective neuronal degeneration in experimental animals with mitochondrial toxins which mimics that seen in HD and which occurs by excitotoxic mechanisms. Beal and co-workers therefore carried out studies with a number of compounds which are selective blockers of the electron transport chain. One of the most interesting of these is the compound 3-nitropropionic acid.

3-Nitropropionic acid (3-NP) is a naturally occurring abundant plant and mycotoxin which has been associated with neurological illnesses in grazing animals and humans (Ludolph *et al.*, 1991). 3-NP is an irreversible inhibitor of succinate dehydrogenase that inhibits both the Krebs cycle and complex II of the mitochondrial electron transport chain (Alston *et al.*, 1977; Coles *et al.*, 1979). Ingestion by livestock results in dyspnea, hindlimb weakness, knocking together of the hindlimbs while walking, and goose stepping (Ludolph *et al.*, 1991). The occurrence of illness in humans has occurred in China after ingestion of mildewed sugar cane, which contains the fungus *Arthrrium*. The illness occurred mostly in children, with the oldest patient presenting encephalopathy being 27 years old (Ludolph *et al.*, 1991). The illness is characterized by initial gastrointestinal disturbance followed by encephalopathy with stupor and coma. Patients who recover have delayed onset of nonprogressive dystonia 7–40 days after regaining consciousness. The patients show facial grimacing, torticollis, dystonia, and jerk-like movements. CT scans show bilateral hypodensities in the putamen and to a lesser extent in the globus pallidus. An earlier report of dystonia following ingestion of mildewed maize may have been the same illness (Woods and Pendleton, 1925).

Studies of mice treated with a single or repeated doses of 120 mg/kg of 3-NP showed depressed motor activity and bilateral symmetric lesions of the caudate-putamen (Gould and Gustine, 1982). In rats repeated doses of 3-NP produced progressive impairment of motor behavior with somnolence, wobbly gait, and finally recumbency with the animals lying on their sides with the hindlimbs rigidly extended (Hamilton and Gould, 1987a). The most consistent lesions were found in the basal ganglia in all affected animals, with some animals showing damage in the CA1 field of the hippocampus and in the thalamus. Ultrastructural studies in both mice and rats showed dendrosomatic swelling, chromatin clumping, and marked mitochondrial swelling, consistent with an excitotoxic injury. The pattern of neuronal injury could not be explained by the distribution of inhibition of succinate dehydrogenase activity, since biochemical and histochemical studies showed it to be uniform throughout the cerebral cortex and basal ganglia (Gould and Gustine, 1982; Gould *et al.*, 1985). The lesions were hypothesized to be due to histotoxic hypoxia, since 3-NP increased arterial blood pressure and arterial blood oxygen compared with controls (Hamilton and Gould, 1987b).

Recent *in vitro* studies showed that 3-NP has no direct depolarizing effects on neurons in hippocampal slices (Riepe *et al.*, 1992), indicating that it does not act at excitatory amino acid receptors. Rather it leads to initial hyperpolarization due to activation of ATP-sensitive potassium channels followed by depolarization due to loss of ATP. Studies in cortical explants showed that 3-NP produces cellular ATP depletion and neuronal damage by a secondary excitotoxic mechanism (Ludolph *et al.*, 1992a). Pathologic changes were significantly attenuated by pretreatment with excitatory amino acid antagonists. In cultured cerebellar granule neurons 3-NP resulted in concentration and time-dependent neurotoxicity (Weller and Paul, 1993). Both MK-801 and the competitive NMDA antagonist 2-amino-5-phosphonovaleric acid delayed but did not prevent the 3-NP toxicity.

Beal and co-workers studied 3-NP neurotoxicity in both rats and nonhuman primates. In initial studies, the effects of intrastriatal injections of 3-NP were examined. These injections produced dose-dependent lesions with neuronal loss and gliosis (Brouillet *et al.*, 1993b). The injections produced ATP depletions at 3 hr, which persisted for up to 24 hr after the injections (Beal *et al.*, 1993a). Using *in vivo* chemical shift magnetic resonance imaging we showed that there were focal accumulations of lactate in the basal ganglia. The lesions were strikingly age-dependent, which was subsequently confirmed (Bossi *et al.*, 1993). This age dependence correlated directly with the increases in lactate following administration of a uniform dose of 3-NP to animals of various ages. The lesions occurred by an excitotoxic mechanism since prior decortication, which removes the striatal glutamatergic input, significantly attenuated the lesions.

Beal and co-workers subsequently examined both subacute and chronic administration of 3-NP in rats. Subacute administration was performed by ip administration of 3-NP over 5–7 days. This resulted in the development of motor slowing and dystonic posturing in the rats. Similar to the observations of Hamilton and Gould (1987a), there were large symmetric lesions in the basal ganglia. With very large lesions there was accompanying neuronal loss and gliosis in the CA1 field of the hippocampus and gliosis in the cerebral cortex. Smaller lesions were confined to the basal ganglia. No lesions were observed in the spinal cord, suggesting that the movement disorder was due to the striatal lesions. Magnetic resonance imaging spectroscopy showed that the lesions were accompanied by focal accumulations of lactate in the basal ganglia which preceded lesions detectable with T₂ weighted imaging, indicating that the energy defect precedes the neuronal degeneration. The lesions were accompanied by a depletion of markers for intrinsic striatal neurons; however, concentrations of the striatal afferent markers dopamine and 3,4-dihydroxyphenylacetic acid were

significantly increased. Histologic studies confirmed a loss of both Nissl and NADPH-diaphorase neurons, yet a sparing of tyrosine hydroxylase immunoreactivity in the striatum.

Systemic 3-NP lesions were also attenuated by prior decortication and by the glutamate release inhibitor lamotrigine. Microdialysis studies showed that there was no significant increase in extracellular glutamate concentrations. These results are consistent with 3-NP causing excitotoxicity by making neurons more vulnerable to endogenous levels of glutamate. Furthermore, *in vivo* ³H-MK-801 receptor autoradiography showed that systemic administration of 3-NP was associated with activation of NMDA receptors (Wullner *et al.*, 1994).

Beal *et al.* (1993a) also examined the effects of chronic low-dose administration of 3-NP subcutaneously for 1 month using Alzet pumps. We reasoned that a chronic low-grade energy disturbance was much more likely to mimic the situation which may occur in neurodegenerative diseases, such as HD. Administration of 3-NP using this paradigm resulted in subtle lesions confined to the dorsolateral striatum, in which there was neuronal loss and gliosis (Beal *et al.*, 1993a). Furthermore, this mode of administration was associated with the sparing of NADPH-diaphorase neurons, similar to findings in HD. A further characteristic feature of HD neuropathology is that there are proliferative changes in the dendrites of spiny neurons, with both recurvatures and increased numbers of spines. Chronic low-grade administration of 3-NP resulted in identical changes in spiny neurons as assessed using the Golgi technique. Wullner *et al.* (1994) also examined mRNA using *in situ* autoradiography. These studies showed a relative sparing of mRNA for somatostatin compared with the amount of mRNA for both substance P and enkephalin. Glial fibrillary acidic protein message was depleted in the center of the largest lesions, but increased at the periphery of the lesions. [³H]Naloxone autoradiography showed a preservation of the striosomal compartmentalization of the striatum. [³H]-Mazindol autoradiography confirmed a preservation of dopamine terminals with lower doses of 3-NP. These studies therefore show that chronic low-dose administration of 3-NP can replicate most of the characteristic neurochemical and neuropathologic features of HD.

Beal and co-workers recently extended their studies to nonhuman primates. The neuroanatomy of the primate basal ganglia much more closely resembles that of man, and it is possible to produce chorea, the cardinal clinical feature of HD. Chronic administration of 3-NP to primates resulted in an apomorphine-inducible movement disorder which closely resembles that seen in HD (Brouillet *et al.*, 1993a). The animals showed orofacial dyskinesia, dystonia, dyskinesia of the extremities, and choreiform movements. Both a clinical rating scale and a quantitative analysis of

tangential velocity of individual movement velocities confirmed that the 3-NP-treated animals had a significant increase in choreiform and dystonic movements.

Histologic evaluation showed that the lesions were strikingly reminiscent of those which occur in HD. There was a depletion of both Nissl-stained and calbindin-stained neurons. In contrast medium-sized aspiny neurons stained with NADPH-diaphorase and large striatal neurons were spared, similar to findings which occur in HD. In addition Golgi studies showed proliferative changes in the dendrites of spiny neurons similar to changes in HD. The patch-matrix compartmentalization of the striatum, which is preserved in HD, was also spared by the lesions. Last, there was sparing of the nucleus accumbens, which is spared in HD. These findings therefore show that chronic 3-NP lesions in primates can produce both an apomorphine-inducible movement disorder and histologic findings which replicate those of HD.

Beal and co-workers have recently carried out studies using magnetic resonance imaging of HD patients *in vivo*. These studies show that there are marked increases in lactate concentrations both in the basal ganglia and in the cerebral cortex (Jenkins *et al.*, 1993). These findings provide direct evidence for impairment of energy metabolism in HD and therefore suggest that 3-NP neurotoxicity which acts through secondary excitotoxic mechanisms may be a relevant model for the disease process.

CYANIDE-INDUCED EXCITOTOXICITY AND NEURODEGENERATION

Cyanide is well known for its rapid, lethal action in which its primary target organ is the central nervous system (Way, 1984). Cyanide rapidly inhibits cytochrome oxidase, which lowers the cell's energy charge and disrupts homeostasis. During cyanide intoxication the appearance of neurological dysfunction occurs within seconds. It has been assumed that the extreme sensitivity to cyanide results from the brain's low anaerobic capacity and limited energy reserve. However, it is becoming increasingly apparent that in addition to disruption of oxidative phosphorylation, cyanide produces other actions in the CNS which can manifest as degeneration of select brain areas.

Several clinical reports have shown that the development of a delayed, progressive Parkinsonism and dystonia can follow acute cyanide intoxication (Uitti *et al.*, 1985; Carella *et al.*, 1988; Grandas *et al.*, 1989; Rosenberg *et al.*, 1989; Valenzuela *et al.*, 1992). In patients surviving a severe, acute intoxication, several days to weeks after the intoxication, dystonia developed which in some cases progressed to a severe Parkinson-like state. Levodopa therapy produced variable improvement. Magnetic resonance imaging showed extensive bilateral lesions of the basal ganglia and

positron emission tomography with 6-fluoro-L-dopa revealed marked dysfunction of dopaminergic transmission in the posterior regions of the basal ganglia, similar to that observed in Parkinsonism (Rosenberg *et al.*, 1989). Also, chronic cyanide exposure has been associated with motor neuron disease, hereditary optic atrophy, and tobacco amblyopia. Kato *et al.* (1985) proposed that a disorder in cyanide metabolism may predispose to motor neuron disease.

Cyanide interacts with several central transmitter systems and altered neurotransmitter function may play a role in cyanide toxicity. Cyanide depletes γ -aminobutyric acid and elevates glutamate in rat brain (Persson *et al.*, 1985). Dopaminergic systems appear to be highly susceptible to cyanide. A lethal dose of cyanide depletes striatal dopamine (DA) in rats (Cassel and Persson, 1992) and local perfusion of cyanide in the striatum stimulates release of DA as measured by *in vivo* microdialysis (Matsumoto *et al.*, 1993). In the PC12 cell model, cyanide produces a calcium-dependent release and depletion of DA and altered DA synthesis and metabolism have been described (Kanthasamy *et al.*, 1991). In rats, lethal doses of cyanide partially inhibit brain DOPA decarboxylase as measured by increased levels of DOPA (Persson *et al.*, 1985). Cyanide also reacts with the DA metabolite 3,4-dihydroxyphenyl acetaldehyde to generate a neurotoxic cyanohydrin (Kanthasamy *et al.*, 1994b). The role of this compound in the cyanide-induced neurotoxic syndrome remains to be determined.

A recently characterized mouse model of cyanide-induced dopaminergic toxicity may prove useful in studying the delayed neurodegeneration produced by cyanide (Kanthasamy *et al.*, 1994a). Following treatment with cyanide over a 7-day period, mice exhibited marked decreases in striatal and hippocampal DA levels which were accompanied by significant peroxidation of lipids in these brain areas. Tyrosine hydroxylase (TH) immunohistochemical examination showed a reduced number of TH-positive cells in the substantia nigra, indicating a loss of dopaminergic neurons. Over one-third of the mice exhibited decreased locomotor activity and akinesia which were suppressed by L-dopa. These behavioral deficits are likely associated with striatal DA deficiency since they are reversed by L-dopa treatment. This animal model is presently being used to study the mechanisms underlying the selective dopaminergic toxicity of cyanide.

Spencer *et al.* (1992) proposed that cyanide is involved in basal ganglia disease through either an excitotoxic effect or disruption of energy metabolism. Defects in mitochondrial oxidative phosphorylation have been associated with Parkinson's disease (Shoffner *et al.*, 1991). Toxicants that inhibit oxidative phosphorylation, including MPTP, carbon monoxide, and manganese, can produce basal ganglia dysfunction associated with locomotor impairment. Cyanide inhibits cytochrome oxidase, the terminal enzyme in mito-

chondrial oxidative metabolism, producing a decrease in cellular ATP levels (Lee *et al.*, 1988). A rapid drop in neuronal ATP would result in altered ionic handling and subsequent breakdown in transmembrane ionic homeostasis. It has been proposed that mitochondria of sensitive brain areas may be highly sensitive to cyanide, resulting in altered bioenergetics and predisposing to neuronal injury.

The role of cytochrome oxidase inhibition as the primary biochemical lesion in cyanide toxicity remains controversial. It is well established that at physiologically active concentrations cyanide inhibits a number of enzymes which are more sensitive to cyanide than cytochrome oxidase (Ardelt *et al.*, 1989). Petterson and Cohen (1985) reported a disparity between the dose-response relationship of cyanide lethality and brain and heart cytochrome oxidase inhibition. Yamamoto (1989) demonstrated in mice rendered unconscious by cyanide that no significant decrease in brain ATP levels occurred. In transverse slices of guinea pig hippocampus, cyanide rapidly depressed synaptic transmission between the Schaffer collateral commissural fiber and CA1 pyramidal cells (Aitken and Braitmen, 1989). This effect of cyanide reversed within seconds of washout, suggesting that cyanide has a direct action on neurons not mediated by metabolic inhibition.

Morphological and biochemical responses to cyanide are related temporally to a rise in cytosolic free Ca^{2+} and in neurons the elevated Ca^{2+} initiates a series of intracellular cascades which can lead to cell dysfunction and death. In electrically excitable cells, destabilization of Ca^{2+} homeostasis by cyanide results from several actions (Johnson *et al.*, 1986). ATP depletion is linked to increased Ca conductance through activation of voltage-sensitive Ca channels (VSCC). In brain the L and N type of voltage-sensitive Ca^{2+} channels may be involved in Ca^{2+} -mediated neural injury. VSCC blockers can attenuate cyanide-induced influx of Ca^{2+} through these channels. Receptor-operated Ca channels play a role in the response since cyanide activates the glutamate/NMDA receptor in primary hippocampal neurons, producing an immediate influx of Ca^{2+} (Patel *et al.*, 1992). This may result from a direct interaction of cyanide with the receptor and/or may be indirectly related to cyanide-stimulated release of glutamate which then activates the receptor. The influx of Ca^{2+} through the NMDA receptor-gated channel contributes to the cytotoxic response to cyanide since death of primary hippocampal neurons is blocked with NMDA receptor blockers (Patel *et al.*, 1993). It is apparent that cyanide can initiate excitotoxicity; its role in cyanide-mediated neuronal degeneration remains to be determined.

The relationship of cyanide-induced cell injury and elevation of cytosolic free Ca^{2+} has been studied in detail. Elevation of neuronal Ca^{2+} triggers intracellular Ca^{2+} -dependent processes and signaling pathways. It appears that when

intracellular Ca^{2+} is increased to excessive levels by cyanide and homeostatic processes are unable to buffer the increase, the ion may activate several critical membranous and cytoplasmic events in concert (Maduh *et al.*, 1990). Unregulated activation of these Ca^{2+} -sensitive reactions can lead to cell dysfunction and eventually, if not controlled, threaten survival of the cell. In a variety of cell types, cyanide-induced activation of endonucleases, phospholipases, and proteases is associated with elevated cytosolic Ca^{2+} . Protease inhibitors attenuate formation of cyanide-induced cell surface blebs, suggesting that cytoskeletal proteins may be substrates for these enzymes. In different cell models, cyanide activates phospholipase A_2 (PLA₂) to produce plasma membrane phospholipid breakdown and mitochondrial dysfunction. In PC12 cells PLA₂ activation by cyanide results from both influx of Ca^{2+} and mobilization of intracellular Ca^{2+} stores (Yang *et al.*, 1994). Cyanide also stimulates generation of inositol triphosphate through an interaction with the glutamate/metabotropic receptor (Yang and Isom, 1994).

Cyanide-induced oxidative stress and subsequent peroxidation of membrane lipids may be important in cellular damage. Exposure of cells to cyanide produces a Ca^{2+} -dependent generation of intracellular hydroperoxides, resulting in lipid peroxidation. Accompanying the generation of hydroperoxides is an inhibition of the brain's antioxidant defense mechanisms (Ardelt *et al.*, 1989). Cyanide inhibits brain catalase, superoxide dismutase, and glutathione peroxidase. In animals intoxicated with cyanide, brain lipid peroxidation is increased and significant lipid peroxidation occurs in the striatum (Kanthasamy *et al.*, 1994a). Oxidative damage to select brain areas appears to play a role in the neurotoxicity of cyanide.

Activation of Ca^{2+} -dependent endonucleases occurs in cyanide-mediated neuronal death. In terminally differentiated PC12 cells, cyanide induces an apoptotic cell death characterized by internucleosomal fragmentation of DNA and chromatin margination which was prevented by an endonuclease inhibitor (Mills and Isom, 1994). It remains to be determined if this form of neuronal death plays a role in the cyanide-induced brain lesions.

Considering the multiple and complex activity of cyanide, it is proposed that cyanide induces a rise in neuronal cytosolic Ca^{2+} which then activates a cascade of biochemical events culminating in the signs, symptoms, and lesions characteristic of cyanide toxicity. Cyanide rapidly depletes energy reserves due to cytochrome oxidase inhibition resulting in an ionic disequilibrium across plasma membranes. Activation of VSCC and NMDA receptors produces a rapid influx of Ca^{2+} which is accompanied by mobilization of intracellular Ca^{2+} stores. Elevated cytosolic free Ca^{2+} initiates a series of intracellular reactions and the neuron eventually loses its homeostatic ability. These Ca^{2+} -

dependent cascades trigger release of neurotransmitters, lipid peroxidation, and activation of destructive enzymes (lipases, proteases, and endonucleases); activation of multiple processes then leads to neuronal injury. In summary, cyanide produces multiple biochemical and functional insults which account for the sensitivity of the CNS to this agent.

PARKINSON'S DISEASE AND EXCITOTOXICITY

Parkinson's disease is one of the most common neurodegenerative disorders, affecting approximately 1% of the population over age 50. Clinical features of the disease include the classic triad of tremor, rigidity, and bradykinesia. From the neuropathologic and neurochemical point of view, Parkinson's disease is characterized by the degeneration of pigmented neurons in the substantia nigra and the depletion of dopamine in the striatum, the area of the brain to which nigral neurons project their axons. The cause of death of dopaminergic neurons in the brain of patients with Parkinson's disease remains unknown, although both genetic and environmental factors have been implicated (Tanner, 1994) and different mechanisms of cytotoxicity have been hypothesized. Among these mechanisms, the one which has attracted the attention of scientists for the longest period of time is oxidative stress (Fahn and Cohen, 1992). More recently, nigrostriatal degeneration has been related to an impairment of mitochondrial respiratory activity (Beal *et al.*, 1993), and pharmacologic and toxicologic evidence has also suggested an involvement of excitotoxicity (Albin and Greenamyre, 1992; Carlsson and Carlsson, 1990). Thus, it appears that no single mechanism may be regarded as the sole cause of neuronal death in Parkinson's disease, whereas, more likely, nigral cell loss may arise from a concerted sequence of toxic events.

In light of these considerations, the following discussion concerning the role of excitotoxicity in Parkinson's disease will emphasize the relationships between EAA-induced damage and other biochemical and pharmacologic alterations. In particular, the mechanisms of action of different neurotoxic agents will be reviewed as models of how excitotoxicity may contribute to nigrostriatal degeneration.

Neurotoxic Models of Parkinson's Disease

As already mentioned, at least three different processes have been suggested to cause neurodegeneration in Parkinson's disease, and, interestingly, the actions of three toxic agents have become prototype models for such neurotoxic mechanisms: namely, dopamine for oxidative stress, MPTP for abnormal mitochondrial activity, and methamphetamine for excitotoxicity. Dopamine not only is the neurotransmitter utilized by neurons in the nigrostriatal pathway, but may also act as an endogenous toxin by generating oxi-

dizing species and thus disrupting the redox equilibrium within dopaminergic neurons. Indeed, dopamine turnover involves its oxidation via monoamine oxidase (MAO), which generates hydrogen peroxide (H_2O_2). Dopamine may also undergo autoxidation with the formation of toxic quinones as well as H_2O_2 (Graham, 1978). The ability of dopamine to induce oxidative stress has been supported experimentally by the work of Cohen and colleagues showing increased levels of oxidized glutathione following pharmacologically induced increased dopamine turnover both *in vivo* and *in vitro* (Spina and Cohen, 1989).

MPTP poisoning mimics most features of Parkinson's disease as closely as anyone could have anticipated at the time of its discovery in 1983 (Langston *et al.*, 1983; Burns *et al.*, 1983). It has therefore become a widely used model for research concerning the nigrostriatal system, providing the initial hint that an impairment of mitochondrial respiratory chain activity could play a role in the pathogenesis of cell loss in Parkinson's disease. MPTP is converted by MAO B to its 1-methyl-4-phenylpyridinium (MPP⁺) metabolite (Chiba *et al.*, 1984), which represents the ultimate mediator of its neurotoxicity (Markey *et al.*, 1984; Langston *et al.*, 1984). MPP⁺ is actively taken up by dopaminergic neurons (Javitch *et al.*, 1985) and it is also accumulated into mitochondria where it acts as an inhibitor of complex I (Ramsay and Singer, 1986; Nicklas *et al.*, 1985). MPTP/MPP⁺ toxicity has been directly linked to a failure of energy supplies in a number of *in vitro* model systems (Di Monte *et al.*, 1986; Denton and Howard, 1987), and, more recently, the depletion of dopamine caused by MPTP in the mouse striatum has been found to be preceded by a selective decrease in ATP levels (Chan *et al.*, 1993b).

The third prototype model for striatal damage is that of methamphetamine toxicity. When administered in repeated doses or in one relatively large dose, methamphetamine causes dramatic damage to nigrostriatal neurons both in rodents and nonhuman primates, as evidenced by long-lasting depletion of striatal dopamine, decreases in tyrosine hydroxylase activity, the number of high-affinity dopamine uptake sites, and histochemical observation of nerve terminal degeneration in the striatum (Ellison *et al.*, 1978; Seiden *et al.*, 1975; Wagner *et al.*, 1980). In 1989, Sonsalla and colleagues reported that the depletion of dopamine and tyrosine hydroxylase activity caused by methamphetamine in the mouse striatum could be prevented by (+)MK-801, an antagonist of the NMDA receptor. This finding, which has since been confirmed and extended by further studies (Muraki *et al.*, 1992; Fuller *et al.*, 1992), not only indicates that EAAs are involved in the mechanism of methamphetamine neurotoxicity, but also supports a role of excitotoxicity in nigrostriatal degenerative processes.

Toxicity models should help us to unravel the precise nature of events underlying a pathologic condition. However,

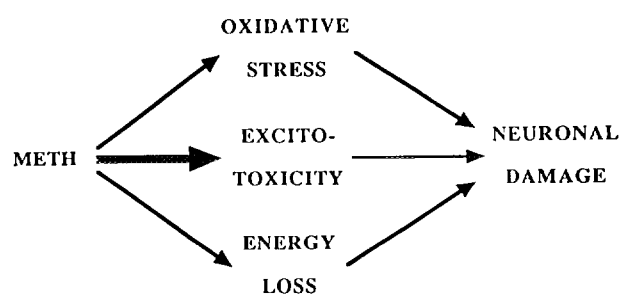


FIG. 2. Schematic representation of the possible mechanism of action of methamphetamine (METH), primarily involving excitotoxicity, with the additional contributions of oxidative stress and energy loss leading to damage of the dopaminergic neurons of the nigrostriatal pathway.

if the mechanisms of toxicity of dopamine, MPTP, and methamphetamine are each viewed as separate models for the pathogenesis of cell loss in Parkinson's disease, more questions are raised than addressed. First, we may question the validity of the models themselves, and then we may still argue about the respective roles that oxidative stress, impairment of mitochondrial function, and excitotoxicity play in Parkinson's disease. Information gathered from the dopamine, MPTP, and methamphetamine models could also lead to a different interpretation, however, namely that an initial insult may then trigger a series of toxic events which ultimately cause nigrostriatal damage. For example, as schematized in Fig. 2 and discussed in greater detail below, methamphetamine neurotoxicity may depend upon EAA-induced alterations, but may also involve other toxic mechanisms.

Excitotoxicity and Nigrostriatal Damage

In addition to excitotoxicity, oxidative stress is likely to be a consequence of methamphetamine exposure. Using intracerebral microdialysis, Nash and Yamamoto (1992) have found an increase in striatal glutamate release after repeated administration of methamphetamine to rats. Glutamate release and, more in general, excitation of the NMDA receptor may result in the production of oxygen radicals (Lafon-Cazal *et al.*, 1993), suggesting one possible link between excitotoxicity and oxidative stress. This link is further supported by the fact that methamphetamine neurotoxicity appears to be strictly dependent upon the presence and release of striatal dopamine which, as previously discussed, may itself lead to oxidative stress. Indeed, methamphetamine causes an increase in extracellular dopamine levels, as assessed by *in vivo* microdialysis (O'Dell *et al.*, 1991), and its toxic effects can be prevented by blocking dopamine synthesis with α -methyl-tyrosine (Gibb and Kogan, 1979).

Recent work has also revealed that a failure of energy supplies may occur after exposure to methamphetamine (Chan

et al., 1994). A significant and rather selective decrease in ATP was found in the striatum of mice injected with methamphetamine. This decrease seemed to be correlated with dopamine depletion, since pretreatment of mice with 2-deoxyglucose, an inhibitor of glucose uptake and utilization, potentiated both ATP and dopamine loss caused by methamphetamine. Taken together, these findings suggest that different toxic events, including excitotoxicity, oxygen radical production, and energy failure, may all play a role in nigrostriatal damage. The theory schematized in Fig. 2 appears therefore to be supported by experimental evidence, making the methamphetamine model particularly valuable not only for studies on the role of excitotoxicity in nigrostriatal degeneration, but also for evaluating the interactions between excitotoxicity and other toxic mechanisms.

If this hypothesis of "interactive" mechanisms of nigrostriatal damage explains methamphetamine neurotoxicity, could it also be applied to the dopamine and/or MPTP models? In particular, is there evidence for a role of excitotoxicity in these models? The interaction between dopamine and EAAs has become a much debated topic in neuropharmacology. It has recently been suggested that dopaminergic terminals in the striatum are part of a corticostriato-thalamo-cortical negative feedback loop, with dopamine release causing the release of EAAs (Carlsson and Carlsson, 1990). It is possible therefore that EAAs may contribute to cell damage in conditions of increased dopamine turnover and, together with oxidative stress, may play a toxic role in the dopamine model of nigrostriatal degeneration.

The involvement of EAAs in the MPTP model is controversial due to conflicting results of studies which have used NMDA antagonists as protective agents against the toxic effects of MPTP (Sonsalla *et al.*, 1989, 1992; Storey *et al.*, 1992; Turski *et al.*, 1991; Zuddas *et al.*, 1992). The reasons for these conflicting data remain to be elucidated, but are likely to include differences in the role of EAAs in various animal species (i.e., rodents vs primates), in various brain regions (i.e., the striatum vs the substantia nigra), and at different time points after exposure to MPTP and/or NMDA antagonists. For example, (+)MK-801 has been found to prevent completely striatal dopamine depletion at early time points (i.e., 1.5, 4, and 8 hr) after administration of MPTP to mice, whereas no protective effect was observed at later times (Chan *et al.*, 1993a). Another factor that may contribute to the action of NMDA antagonists as well as to the conflicting data on their effects on MPTP neurotoxicity is the fact that (+)MK-801 has been reported to modify the rate of elimination of MPP⁺ from the mouse striatum (Chan *et al.*, 1993a).

Although the interactions of NMDA antagonists with MPTP in different experimental conditions are not completely clear, the overall evidence presented to date makes

it conceivable that excitotoxicity participates in the cascade of MPTP-induced toxic events. In particular, it is possible that increased extracellular levels of EAAs may result from the failure of energy supplies caused by MPP⁺ accumulation into mitochondria and the consequent inhibition of oxidative phosphorylation. Indeed, energy impairment may lead to decreased reuptake and/or increased release of EAAs (Nicholls and Atwell, 1990). The hypothesis of a relationship between excitotoxicity and mitochondrial abnormalities, supported by the MPTP model, has gained wide interest among neuroscientists not only as a mechanism for nigrostriatal damage, but also as a more general feature of the pathogenesis of neurodegenerative disease (Albin and Greenamyre, 1992; Beal, 1992).

Implication for Parkinson's Disease

Two main conclusions can be drawn from the dopamine, MPTP, and methamphetamine models of neurotoxicity, namely that (1) a series of insults rather than a particular toxic mechanism is likely to underlie nigrostriatal degeneration and that (2) these insults are likely to involve excitotoxicity, which could either represent an initial primary event or the consequence of other neurotoxic changes. These conclusions are compatible with findings of studies on Parkinson's disease, providing evidence in favor of the occurrence of oxidative stress, mitochondrial abnormalities, and excitotoxicity.

Oxidative stress has been implicated in Parkinson's disease because of findings in the substantia nigra of patients showing, for example, (1) increased levels of nonheme iron (Dexter *et al.*, 1989b) as well as products of lipid peroxidation (Dexter *et al.*, 1989a) and (2) impaired antioxidant defense mechanisms in the forms of decreased levels of reduced glutathione (Perry *et al.*, 1982) and decreased activities of glutathione peroxidase (Kish *et al.*, 1985). Defects in oxidative phosphorylation have also been found in different tissues of patients, leading to the hypothesis that Parkinson's disease may be a mitochondrial disorder (Di Monte, 1991). While data concerning a possible failure in mitochondrial respiratory chain activity in the skeletal muscle remain controversial (DiMauro, 1993), more consistent results have been obtained by comparing specimens from the substantia nigra of patients vs control subjects (Schapira *et al.*, 1990; Mizuno *et al.*, 1994). Indeed, abnormalities in the nigral tissue appear to be rather selective for mitochondrial complex I. Evidence supporting a role of both oxidative stress and mitochondrial impairment in Parkinson's disease should not be surprising not only in view of our previous discussion, but also because mitochondria represent a primary target for oxidative damage and, vice versa, because impairment of mitochondrial activity may itself generate oxidizing species (Di Monte *et al.*, 1992).

The occurrence of EAA-induced damage in the Parkin-

sonian brain is relatively less documented, although initial studies using animal models of Parkinsonism have already led to pilot clinical trials of NMDA antagonists as antiParkinsonian drugs (Montastruc *et al.*, 1992). It has been shown, for example, that (1) NMDA blockers potentiate the antiParkinsonian action of levodopa (Klockgether and Turski, 1990), (2) that (+)MK801 stimulates locomotor activity in mice previously depleted of monoaminergic stores (Carlsson and Carlsson, 1990), and (3) that microinjections of EAAs into different areas of the basal ganglia of rats cause electromyographic activity reflecting Parkinsonian rigidity (Klockgether and Turski, 1993). It is also noteworthy that the role of EAA receptors may vary depending upon receptor subtypes and sites within the basal ganglia (Klockgether and Turski, 1993), suggesting that nigrostriatal damage may be the result of molecular changes more complex and selective than previously thought and emphasizing the need for more detailed pharmacologic and toxicologic studies.

The high selectivity of EAA-mediated neurotransmission may contribute to an essential feature of neurodegenerative disorders such as Parkinson's disease, namely their discrete and characteristic patterns of neuronal death. Such selectivity may be further enhanced if EAA-induced cell damage is associated with other neurotoxic mechanisms which may themselves target specific populations of neurons. Other critical features of neurodegenerative disorders are their delayed onset and gradually progressive evolution. Both of these characteristics may result from the development of metabolic abnormalities which may render neurons progressively more susceptible to excitotoxicity. For example, a failure of energy supplies may become increasingly evident as a result of the aging process (Di Monte *et al.*, 1993) and may "predispose" to the toxic effects of EAAs (Henneberry *et al.*, 1989). Thus, excitotoxicity could explain a number of features of neurodegenerative disorders, leading to the hypothesis that it may represent the final common pathway of neuronal death (Albin and Greenamyre, 1992). This hypothesis also points to alterations in energy metabolism as the most likely mechanism which would enhance the susceptibility to EAA toxicity as well as the selectivity of neuronal damage (Beal, 1992). A similar scenario seems particularly plausible for Parkinson's disease, consistent with findings from the Parkinsonian brain as well as from the toxic models of nigrostriatal damage reviewed in this paper.

SUMMARY

A central theme that emerges from this review is that excitotoxicity is inextricably intertwined with neuronal bioenergetics and mitochondrial function. Neurotoxicants that disrupt cellular energy metabolism may interact synergistically with age-associated decrements in glucose utilization

and mitochondrial function. Oxidative stress and reduced intracellular energy levels can interact to increase both extracellular GLU concentrations and GLU receptor-mediated excitotoxicity (Coyle and Puttfarcken, 1993; Henneberry *et al.*, 1989). Age-related or genetic alterations in antioxidant defense mechanisms and/or mitochondrial function may render individuals susceptible to a variety of environmental neurotoxins.

Three major points clearly emerge from the integration of the various perspectives presented here: first, the value of using neurotoxicants as models for elucidating the pathogenic mechanisms underlying both rare and common neurological diseases; second, the high degree of overlap that exists between prooxidant and excitatory events; and last, the fact that, while many diverse agents and diseases can cause selective damage to the nervous system, these specific pathologies may be underlaid by a few ubiquitous vulnerable features found in all neural tissues. Excitatory amino acids acting as final common mediators of neuronal death appear to be one of those vulnerable features that interact with both pre- and postsynaptic mechanisms that are altered by the aging process.

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