Differential Vulnerability to Excitatory Amino Acid-Induced Toxicity and Selective Neuronal Loss in Neurodegenerative Diseases

John H. Weiss and Dennis W. Choi

ABSTRACT: Neurodegenerative diseases are characterized by selective degeneration of certain biochemically distinct subpopulations of central neurons. Studies of the intrinsic vulnerability of such neurons to injury by excitatory amino acids in vitro, as well as study of neurologic syndromes produced in animals or humans by ingestion of environmental excitatory amino acid neurotoxins may suggest a link between excitotoxicity, and the pathogenesis of certain neurodegenerative diseases.

RESUME: Vulnerabilité différentielle à la toxicité induite par des acides aminés excitateurs et perte neuronale selective dans les amladies neurodégneratives. Les maladies neurodégneratives sont caractérisées par une dégénérescence sélective de certaines sous-populations de neurones centraux biochimiquement distinctes. Des études de la vulnérabilité intrinsèque de ces neurones à une atteinte par des acides aminés excitateurs in vitro, ainsi que l’étude des syndromes neurologiques causés chez l’animal ou chez l’humain par l’ingestion de neurotoxines environnementales qui sont des acides aminés excitateurs, peuvent suggérer qu’il existe un lien entre l’excitotoxicité et la pathogénèse de certaines maladies neurodégneratives.

There is now substantial evidence that excitatory amino acid-induced neurotoxicity (excitotoxicity) can contribute to the neuronal injury triggered by acute insults to the central nervous system.1,2 In part based on this precedent, there has been growing recent interest in the possibility that excitotoxicity might also participate in the pathogenesis of the selective neuronal loss accompanying certain neurodegenerative diseases, including Huntington’s disease (HD), Alzheimer’s disease (AD), and amyotrophic lateral sclerosis (ALS).

While glutamate activates both N-methyl-D-aspartate (NMDA), and non-NMDA (kainate, and alpha-amin0-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA]/quisqualate) receptors,3 the excitotoxicity associated with several acute insults to the central nervous system may be predominantly mediated by NMDA receptor activation. This predominance may reflect a key role of NMDA receptors in mediating the degeneration of cortical or hippocampal neurons induced by brief exposure to high concentrations of glutamate.4,5 Yet, with prolonged exposures, non-NMDA agonists are also potent neurotoxins and can produce widespread neuronal destruction.6

The injection of kainate into rat striatum destroys intrinsic striatal neurons with sparing of afferent axons, a lesion sharing some features with the pathology of HD.7,8 In 1985, Ferrante et al.9 discovered that the degeneration of striatal neurons in HD was strikingly selective, in that the small neuronal subpopulation containing high concentrations of the enzyme, NADPH-diaphorase (NADPH-d(+) neurons), remained largely intact amid extensive loss of other intrinsic striatal neurons. This new pathological feature challenged the excitotoxin injection model, and Beal et al.10 found that kainate failed to spare NADPH-d(+) neurons. In fact, of several excitotoxins studied by those investigators, only the NMDA agonist quinolinate successfully mimicked the pattern of striatal neuronal loss sparing NADPH-d(+) neurons, favoring the idea that quinolinate might be specifically involved in HD pathogenesis.10,11 However, other investigators did not see NADPH-d(+) neuronal sparing with quinolinate injection.12,13

We set out independently to characterize the excitotoxic vulnerability of cultured cortical NADPH-d(+) neurons, a population sharing the property of somatostatin co-localization with striatal counterparts. We reasoned that NADPH-d(+) neurons might be intrinsically resistant to excitotoxicity, although such intrinsic resistance would only be one of several possible explanations of resistance to excitotoxicity in vivo. The damage induced by administration of an excitotoxin in vivo might additionally reflect cellular access, uptake, and the extent of secondary damage induced by release of endogenous excitatory amino acids. In the open architecture of cell culture, the first two factors are attenuated, and intrinsic neuronal vulnerability can be quantitatively assessed.

From the Department of Neurology H-3160, Stanford University Medical Centre, Stanford
Reprint requests to: Dennis W. Choi, Department of Neurology H-3160, Stanford University Medical Centre, Stanford, California, U.S.A. 94305
Consistent with the results of Beal et al., we found that cultured cortical NADPH-d(+) neurons, and their striatal counterparts, were selectively resistant to quinolinolate neurotoxicity; however in contrast, we found that this selective resistance generalized to other NMDA agonists. Since this resistance was not absolute, it seemed plausible that divergent results in vivo might reflect small differences in technique and resultant differences in the concentration profiles of excitotoxin attained. Indeed subsequently Beal et al. reexamined striatal lesions induced by injection of submaximal amounts of NMDA, and found NADPH-d(+) neuronal sparing that they had not seen previously.

An attractive site for explaining the resistance of NADPH-d(+) neurons to damage by NMDA agonists might be at the receptor level (e.g., a paucity of NMDA receptors, or reduced receptor affinity for NMDA or glycine). NADPH-d(+) cells are not simply hard to kill, as they are preferentially destroyed by kainate or quisqualate. Additional support for involvement of NMDA receptor-mediated injury in HD was provided by the observation that another neuronal population spared in HD, neurons containing acetylcholinesterase, is also resistant to NMDA receptor-mediated injury; and the finding that NMDA receptor number may be greatly reduced in HD striatum.

The possibility that excitotoxicity may participate in the pathogenesis of ALS has been raised by suggestions of aberrant glutamate metabolism in ALS patients. Precedent is provided by three environmentally-induced motor system diseases, lathyrism, the ALS-Parkinsonism-dementia complex of Guam (ALS-PD), and domoate poisoning. Lathyrism is characterized by spastic paraparesis; it is caused by excessive consumption of a potent and selective agonist at non-NMDA receptors. A recent binding study has also suggested denervation of muscle consistent with damage to motor neurons or axons. Pathological correlates of this denervation will be an important topic for future investigation.

The apparent ability of several non-NMDA agonists - BOAA, BMAA (at least experimentally), and domoate - to damage the primate motor system supports the hypothesis that non-NMDA receptor overstimulation might contribute to the pathogenesis of sporadic ALS. Furthermore, electrophysiological studies have suggested that motor neurons are highly sensitive to kainate, perhaps more so than to NMDA; and intrathecal injection of kainate has been recently found to preferentially damage neurons in ventral horn.

Excitotoxicity mediated through NMDA receptors has also been proposed to participate in the pathogenesis of AD, based on several arguments including distribution of pathology. Whether or not NMDA receptors are preferentially lost has been controversial. We have raised the alternative speculation that any excitotoxic involvement in AD may be specifically mediated via non-NMDA receptors. The small subpopulation of cortical neurons containing somatostatin (SS), which as a population overlap substantially with the NADPH-d(+) population, may be selectively damaged in early AD. Another cortical neuronal subpopulation possibly sustaining early damage in AD may be neurons containing high concentrations of the calcium binding protein, parvalbumin (PV). Both SS (+) and PV (+) neurons are relatively resistant to NMDA receptor-mediated toxicity and unusually sensitive to non-NMDA receptor-mediated toxicity. Whatever the cause of Guam ALS-PD, the concurrence of AD-like features with ALS-like features in that disease raises the possibility that AD may share some mechanisms with ALS, perhaps including non-NMDA receptor-mediated toxicity. Glutamate-induced degeneration of cultured hippocampal neurons is associated with increased immunostaining with antibodies directed against AD neurofibrillary tangles.

Many different specific derangements in the glutamate system could eventually lead to the toxic overactivation of NMDA or non-NMDA receptors in a neurodegenerative disease. These derangements need not be primary disease manifestations, for example a direct effect of the HD gene, but could come about secondarily after a cascade of earlier pathological events. An excitotoxic pattern of neuronal loss would result as long as excitotoxicity contributed importantly to net injury.

Increases in the ambient concentrations of glutamate or other endogenous excitatory amino acid, such as aspartate, homocysteate or quinolinate, could result from increased production or release, or deficient uptake or metabolism. Another endogenous excitotoxin is the amino acid cysteine, that like BMAA ingestion can damage upper and lower motor neurons. Despite lacking the side-chain electronegative group characteristic of other excitatory amino acids, BMAA can induce seizures in rodents, and excitotoxic neuronal degeneration in CNS explant tissue, most likely, extracellular bicarbonate/CO₂ interacts with BMAA to produce a combined structure capable of activating glutamate receptors - possibly after carbamate formation. Although the neurotoxicity induced by high concentrations of BMAA may be largely mediated by NMDA receptors, low concentrations of BMAA selectively destroys NADPH-d(+) neurons and thus may preferentially activate non-NMDA receptors. A recent binding study has also suggested that BMAA interacts with non-NMDA receptors.

Domoate is a potent kainate agonist. Domoate poisoning, due to ingestion of contaminated mussels, has been recently recognized as a clinical entity characterized by seizures and other neurological disturbances; of note, electrophysiological studies have suggested denervation of muscle consistent with damage to
The postulate of glutamate receptor-mediated neurotoxicity in the pathogenesis of HD, ALS OR AD suggests a possible avenue for therapeutic intervention. Specific agents might be directed at either presynaptic or postsynaptic sites, for example decreasing glutamate release, blocking glutamate receptors, reducing excitotoxic amplification, or blocking the expression of excitotoxicity downstream from receptor overactivation. Given the lack of good animal models for these diseases, clinical trials with suitable anti-excitotoxic agents may be the only way to determine the extent to which excitotoxicity contributes to pathogenesis. A key task for the future will be the development of anti-excitotoxic agents sufficiently free of side effects to be suitable for long-term administration in humans.

ACKNOWLEDGEMENTS

Supported by NIH grant NS26907 and a Clinician Investigator Award (# AG00495-01, to J.H.W.) from the NIA.

REFERENCES

15. Koh J, Choi DW. Cultured striatal neurons containing NADPH-diaphorase or acetylcholinesterase are selectively resistant to injury by NMDA receptor agonists. Brain Res 1988; 446: 374-378.
51. Mattson MP. Antigenic changes similar to those seen in neurofibrillary tangles are elicited by glutamate and Ca2+ influx in cultured hippocampal neurons. Neuron 1990; 2: 105-117.