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Neurotoxic Amino Acids and Structurally Related Analogs

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I. Introduction

For at least 1000 years, the Chinese have used an extract of a common seaweed, *Laminariae Japonicum,* as an additive to their cooking to enhance the flavor of many different types of foods. In 1910, the agent responsible for the flavor-enhancing effects of these seaweed extracts was found to be glutamic acid (52). Since that time the production of glutamic acid has been industrialized and this compound has been added in ever increasing quantities to enhance the flavor of many natural and artificial food stuffs. In recent years it has been discovered that extracts of other seaweeds and plants may contain even more potent taste enhancers than monosodium glutamate (MSG), which are widely abundant in nature.

In the early 1970s the dicarboxylic amino acids, such as glutamic acid and several of the structurally related heterocyclic taste enhancers, began to attract scientific attention when it was discovered that these compounds were neurotoxic when administered in large parenteral doses to young rodents (51, 69). Subsequently, both scientific and lay interest intensified following the demonstration of their neurotoxic potential after oral administration (10, 49, 76). The culmination of this interest has been the discontinuation of the addition of monosodium glutamate to infant and baby foods. In today's atmosphere of caution concerning the possible role of food additives, preservatives and flavor enhancers in mental retardation, the question of use of monosodium glutamate has greatly added to the controversies surrounding these issues. Thus, it seems timely to review the neuropharmacology of the neurotoxic amino acids, the evidence supporting their neurotoxicity and the recent interest in these compounds as research tools in the field of neurochemistry.

II. Historical Perspectives

Glutamic acid has always been of great interest to neurochemists and, at one time, it had even been suggested that glutamic acid was the only amino acid which could be metabolically useful to the central nervous system (125). Based on these experiments, it was assumed that glutamic acid might be of metabolic use to an injured

brain and as late as 1951 large doses of monosodium glutamate (20–30 g/day) were still being explored for the treatment of mongoloid idiocy (127), retardation (58) and seizure disorders such as petit mal (93). In addition, studies were even carried out to determine whether daily doses of MSG of this magnitude might serve to enhance learning in normal children (58). It was in this setting that in 1957, Lucas and Newhouse (51) began to investigate the protective effect of L-glutamic acid on hereditary retinal degeneration of rats. They discovered that far from being protective against the hereditary neurodegeneration that they were studying, large amounts of monosodium glutamate resulted in irreversible destruction of the majority of cells in the inner layer of the retina within minutes to hours after its parenteral administration (51). This initial observation was subsequently confirmed by other authors (14, 70), and in 1969 Olney (69, 71) discovered that the neurotoxicity of large parenteral doses of monosodium glutamate in rodents was not restricted to the retina but also appeared to involve the medial basal hypothalamus (particularly the perikarya of the arcuate nucleus). After this observation several investigators (3, 87), using somewhat modified protocols, attempted, but failed, to repeat Olney's initial experiments.

In the following years, however, others were able to substantiate Olney's findings and it was subsequently demonstrated that MSG was neurotoxic not only following large parenteral doses but also after oral administration (1, 10, 49, 76). Later, several other dicarboxylic amino acids and related straight-chain and heterocyclic amino acids were found to exhibit the so-called "glutamate retino-hypothalamic neurotoxicity." From this developed the concept of a class of potent, ubiquitous, naturally occurring neurotoxic amino acids which might be as of yet unrecognized pathogenic significance in human mental retardation syndromes and developmental learning disorders *vide infra* (60, 74, 77, 79, 82, 103).

A list of those amino acids that have been found to be neurotoxic and their order of potency is given in Table 1. It should be emphasized that, although most of the following discussion deals with evidence obtained using glutamic acid as the experimental model (since this compound has been the most intensively studied), experience with other related analogs suggests that it is not invalid to generalize from these particular experiments to conclusions concerning the neurotoxic amino acids as a class.

III. Histology of Retinal Lesions

Following large doses (1–4 mg/g body weight) of MSG or others of the neurotoxic amino acids given parenterally to susceptible species such as the rat and the mouse, there occurs a degenerative lesion in the inner layer of the retina involving the majority of amacrine, ganglion and bipolar cells. The outer layer, in particular the photoreceptors, however, is spared (13, 51, 70).

TABLE 1
Order of potency of the neurotoxic amino acids and related analogs

	Potency[a]
Kainic acid	200–500
Ibotenic acid[b]	200–500
Quisqualic acid[b]	200–500
N-Methyl-DL-aspartic acid	100
DL-Homocysteic acid[c]	20
β-N-oxalyl-L-α, β-diamino propionic acid[c]	20
Cysteine-S-sulfate[b]	20
N-Methyl-DL-glutamic acid[b]	5
L-Glutamic acid	1
D-Glutamic acid[c]	1
L-Aspartic acid	1
D-Aspartic acid	1
L-Cysteine sulfinic acid[c]	1
L-Cysteine sulfonic acid	1
L-Cysteic acid[c]	1
L-Cysteine[b]	1

[a] Relative potency of the neurotoxic amino acids. Based on dosages in mmol/kg (Data abstracted from References 74, 77, 80, 109).

[b] Analogs for which data on oral efficacy are not available.

[c] Analogs which appear to have little or no oral efficacy.

TABLE 2
Age dependency of toxicity of L-glutamic acid

Age (days)	LED[a] (mg/g body wt)	
	(Oral)	(s.c.)
10	0.50	0.35
21	1.00	0.80
45	1.50	1.20
60	2.00	1.50

[a] Lowest effective dose of systemically administered L-glutamate (Table taken from Reference 74, with permission of publisher and author).

Although it is possible to produce lesions of the retina in the adult rodent, such lesions are usually partial and unpredictable and associated with high mortality rates characterized by extensive and recurrent seizure activity (70). Lower doses given orally or parenterally to neonatal rodents such as the mouse appear to be much more effective and induce extensive and predictable patterns of retinal degeneration (13, 70). In general, the neonatal mouse maintains this rather marked susceptibility to the neuroretinotoxic effects of MSG up until about 1½ to 2 weeks of age, when an increasing resistance to the effects of the drug appears to develop, indicating an increasing threshold to the neurotoxic amino acids with aging (70) (Table 2). As determined by light and electron microscopy, histological evidence of the neurotoxic effects of MSG can be demonstrated within minutes to hours after acute administration of the compound (13, 51, 70). Cell soma and dendritic processes become swollen and develop frank necrosis within 2 to 3 hr (13, 51, 70). None of the cytopathological changes that have been observed are specific for any of the cells. Furthermore, very few of the cells in the inner retinal layer survive and destruction is complete within a few hours (70). Subsequently, macrophages (probably microglia) (48) and other phagocytic cells enter the area and begin the process of neuronophagia (79). Glial and supporting cells are not irreversibly damaged and there appears to be no primary effect of MSG upon the vascular supply to the retina itself (70). That the retinal degeneration induced by monosodium glutamate is not solely an *in vivo* phenomenon is evidenced by the direct retinal toxicity of MSG on chick embryo retinal cultures (99). Further evidence for a direct cytotoxic effect of the neurotoxic amino acids is a similar pattern of destruction of the inner layer of the retina following the direct intraocular injection of kainic acid, a heterocyclic glutamic acid analog (Table 1) (106).

IV. Histology of Extraretinal Lesions

In addition to the retina, that area of the central nervous system which seems to exhibit the greatest sensitivity to the neurotoxic effects of parenteral and oral monosodium glutamate appears to be a region of the medial basal hypothalamus largely restricted to the arcuate nucleus of the hypothalamus (10, 46, 49, 69, 74, 76, 78, 113, 117). It should be emphasized that in addition to glutamic acid, all of the other straight-chained and heterocyclic amino acids listed in Table 1 are neurotoxic when given parenterally in large amounts to neonatal chicks, hamsters, guinea pigs, rabbits, rats and mice (60, 73, 74, 77, 79, 82, 103). Not all of these compounds, however, are neurotoxic after oral administration. This difference in oral potency is undoubtedly a result of differences in inactivation and uptake within the intestines. Histologically, the time of onset and evolution of the lesion in the arcuate nucleus of the hypothalamus are very similar to those of the inner layer of the retina (10, 49, 69, 71). All cell types within the vicinity of the arcuate nucleus appear to be affected, with ependymal, glial and neuronal cells showing acute swelling and intracellular edema (10, 49, 71). There is no consistent change in the neuropil of this area (10, 49, 71). Within 5 to 10 hr of the administration of a dose of monosodium glutamate to a neonatal rodent, necrosis of dendrites and soma of arcuate neurons appears to be complete. Subsequently, there is neuronophagia with macrophage [probably microglia (48)] infiltration (10, 49, 71).

All of the acute intracellular changes observed in glia appear to be reversible and there is no lasting destruction of these elements within the medial basal hypothalamus (10, 49, 71). Examination of the arcuate nucleus both acutely and when the animals have reached adulthood suggests that the neurotoxicity of these compounds is limited solely to perikarya lying within the arcuate nucleus and perhaps the ventral medial aspect of the ventromedial nuclues, whereas glial cells, ependymal cells and axons of passage are spared (83). There appears to be no primary destruction of the vascular endothelium or vascular supply to the area (49). It has been suggested that the topographical evolution of the lesion in the arcuate nucleus proceeds from the ventricular surface centrifugally, implying diffusion of the toxic amino acid into the neuropil of the arcuate nucleus from cerebrospinal fluid (49). Whether the cerebrospinal fluid is in fact the portal of entry for the neurotoxic amino acids has not been proven, and other evidence suggests that such a hypothesis may be incorrect (*vide infra*).

It was initially believed that the neurotoxicity of systemically administered glutamic acid was limited to the arcuate nucleus of the hypothalamus but later more thorough studies have indicated that, although the arcuate nucleus appears to be the most sensitive CNS area, higher doses of the neurotoxic amino acids induce diffuse lesions in more extended areas of the central nervous system (49, 82). Further study has also suggested that the arcuate nucleus is not alone in its peculiar susceptibility to the neurotoxic effects of systemically administered MSG (69, 81). The organum vasculosum lamina terminalis, subcommissural organ, subfornical organ and the area postrema as well as the areas surrounding them also appear to possess a unique susceptibility to the toxic effects of MSG and its related analogs (49, 69, 81). It is interesting to note that all of these structures including the median eminence comprise a set of similar structures, the circumventricular organs, in which unique anatomical features provide an easy communication between the resident neuropil and the systemic circulation (43). Because of their loosely fenestrated capillary endothelium, the circumventricular organs have been considered to possess no local blood brain barrier, and it is quite probable that this unique feature is the basis of the apparent anatomical selectivity of peripherally administered neurotoxic amino acids for these structures (43, 81). Such a proposal would not be inconsistent with the histological evolution of the lesion described above.

As is true for the retinal degeneration induced by monosodium glutamate and its analogs, the neonatal animal appears to be more susceptible to the neurotoxic effect of these compounds than does the adult animal, and its susceptibility appears to wane after 10 days of age (49, 65, 71) (Table 2). Nevertheless, it should be emphasized that in rodents such as the mouse the administration of moderately high doses of MSG (1–4 mg/g body weight) produces unequivocal damage in the adult, although the number of involved cells and area of damage may be less consistent than that observed following a similar dose in neonatal animals (49, 65, 71). Adult rats appear to be relatively resistant to the toxic effects of parenteral and oral glutamate (65, 71, 74). It is not clear whether species other than those described above are susceptible to the neurotoxic effects of peripherally administered glutamate. Although it was initially suggested that the rhesus monkey (*Macacca mulatta*) was sensitive to the neurotoxic effects of glutamate (84), other authors have failed to corroborate these findings, suggesting that the susceptibility to glutamate may be peculiar to rodents and lower species (1, 2, 101, 116).

As alluded to previously, one of the more dramatic pharmacological effects of the neurotoxic amino acids is their ability to induce sustained and recurrent seizures in both neonatal and adult animals (8, 51, 79). The species specificity for these induced seizures has not been adequately explored.

As shown in Table 1 the potencies of the

neurotoxic amino acids are quite varied, with the toxicity of the well studied glutamate being perhaps some 200- to 500-fold less than that of some heterocyclic isoxazole analogs such as kainic and ibotenic acids (74, 109). The potency of kainic acid is so great that the subcutaneous administration of neurotoxic doses usually results in continuous seizures and death within 2 to 3 hr (60, 82). Although listed in the table as one of the glutamate-like neurotoxic amino acids, it is not clear whether the neurotoxicity of cysteine is comparable to that of glutamate and its other related analogs (60, 74, 77, 79). In sublethal doses cysteine produces a diffuse and widespread neurodegeneration which, although beginning in the arcuate nucleus, involves many brain areas in a pattern completely different than that of glutamate (79). The reason for this difference is not clear but may well result from toxicity of a metabolite of cysteine produced diffusely throughout the central nervous system (*vide infra*) (79).

V. The MSG Syndrome

Animals that have received monosodium glutamate as neonates and that survive to adulthood manifest several obvious endocrinological, metabolic and behavioral abnormalities, which appear to be at least partially secondary to the neurotoxic effects of MSG (5, 9, 32, 46, 63, 66, 67, 69, 83, 88, 92, 96, 117, 123). The animals are stunted and obese, display abnormal estrous cycles, are relatively infertile and possess smaller pituitaries, ovaries and uteri than controls (9, 32, 46, 63, 66, 67, 69, 83, 96, 117). Adult animals that have received glutamic acid as neonates demonstrate increasing irritability and tail automutilation (66). In addition, animals so treated demonstrate behavioral deficiencies in spontaneous alternation tasks, maze tests, pattern discrimination and open field activity (5, 88, 92, 123). They may demonstrate decreased spontaneous motor activity as well as deficiencies in discrimination learning (66, 92). Others have suggested that there is an increase in spontaneous activity associated with monosodium glutamate treatment of mice (5). Feeding patterns appear to be normal, and, although obese, the animals do not appear to be hyperphagic (66). In addition, careful behavioral testing suggests that the animals are blind (G. N. Ervin, C. B. Nemeroff and J. S. Kizer, unpublished observations), a finding paralleling their gross optic atrophy (13). Thus, it appears clear that the neurodegenerative lesions induced by the neurotoxic amino acids have grave consequences for the animal as an adult.

VI. Mechanisms of Neurotoxicity

There are two issues relevant to the mechanism of the neurotoxicity of the glutamate-related analogs. One is the manner in which peripherally administered glutamate exerts a selective toxicity in the regions of the circumventricular organs, and, second, the cellular events leading to neuronolysis and death while axons of passage and glia are spared. Not long after the initial discovery that peripheral MSG was neurotoxic, it was discovered that the intracerebral injection of either glutamate or kainic or other analogs produce typical lesions at the injection site (16, 75, 112, 121). Histologically, these lesions are identical to those of the arcuate nucleus seen after peripheral administration of these compounds and are characterized by neuronolysis, but sparing of glia and neuropil in the vicinity (16, 112, 121). Such evidence also argues against the neurotoxicity of MSG as being secondary to a primary vascular event. It appears that a large majority of cells within the central nervous system are sensitive to glutamate and its analogs, whereas certain cells, such as the granule cells within the cerebellum and perhaps others, are unaffected (14) (*vide infra*). Therefore, it is reasonable to assume that the anatomical distribution of neurotoxic effects of peripherally administered MSG is a result of unique regional differences in the blood-brain barrier, which make the circumventricular organs and their surrounding tissue much more permeable to the neurotoxic amino acids.

The differential sensitivity of neonatal and adult animals is also intriguing. There is no apparent difference in sensitivity of adult and neonatal animals to direct intracerebral injection of a neurotoxic amino acid, although this point has by no means been clearly demonstrated. Some of the differences in sensitivity to peripheral administration of neurotoxic amino acids may involve maturation of regional blood-brain barriers, age-induced changes in neuronal dendritic and somal processes, or changes in the ability of the liver to metabolize glutamate.

It is important to note that the molecular mechanisms for the neurotoxicity of these compounds do not involve an excessive cationic or osmotic load. Injections of saline, sodium glutarate, glycine, serine, proline, leucine, arginine or lysine, which are isotonic with sodium glutamate, do not reproduce the glutamate pattern of neurotoxicity (47, 74, 76). These findings and the demonstration that small amounts of other neurotoxic amino acids (1–2 μg) injected intracerebrally are neurotoxic makes it clear that the neurotoxic effects are a direct result of the anionic backbone of glutamate and its related straight-chain amino acid and heterocyclic analogs. That the neurotoxicity of these compounds involves a direct interaction with the neuron and does not result from a temporary destruction of glial and supporting elements is supported by the finding that the peripheral administration of *alpha* amino adipic acid does not result in irreversible destruction of neuronal elements, although glia are permanently destroyed (77). Therefore, the conclusion seems warranted that the neurotoxicity of the glutamate-like analogs results from direct interaction of these compounds with the cell body of the neuron.

Several lines of evidence indicate that the neurotoxic event may occur following contact of the neurotoxic amino acid with the outer membrane of the neuron.

1) Both enantiomorphs of many of the neurotoxic straight-chain amino acids are equipotent even though they are not metabolized (74, 77).[1]

2) N-acetyl-D-aspartate, homocysteate and ibotenate do not block glutamate uptake in brain slices, indicating that neuronal uptake by a glutamate transport system is not necessary for expression of their neurotoxicity (6).

3) Intraneuronal injection of glutamate into spinal motor neurons has no electrophysiological effects (14).

4) The distribution or topography of those cells which are sensitive to glutamate and its analogs seems to parallel the distribution of glutamate receptors, both in the spinal cord and in the central nervous system (21, 25, 29, 50, 54, 75).

5) Cells that appear to lack glutamate receptors, such as the granule cells of the cerebellum, appear to be resistant to the neurotoxic effects of these compounds (16, 29).

6) Blockade of uptake of glutamic acid by organometal compounds enhances neuronal sensitivity to the electrophysiological effects of glutamate (18).

7) The susceptibility to kainic acid of neuronal aggregates in culture increases with age, perhaps due to a maturation of glutamate-like receptors (34).

Thus, these several lines of evidence appear to support a direct outer membrane as the basis for their neurotoxicity.

Several years before the demonstration of the neurotoxicity of glutamate and its related analogs, Curtis and Watkins (18, 20) had carefully defined a group of neuroexcitatory amino acids (Table 3) and examined their ability to depolarize or excite spinal motor neurons (20). Subsequently, the heterocyclic analogs, kainic, quisqualic, tricholomic, domoic and ibotenic acids were also demonstrated to be powerful neuroexcitants, which like MSG were capable of inducing convulsions and stimulating spinal

[1] Dextrorotary optical forms of the amino acids such as glutamate are not believed to undergo intermediary metabolism, although it is clear that active uptake and metabolism of D-glutamate to 5-oxoproline does occur (57). It may well be that this capacity to metabolize D-amino acids such as glutamate evolved as a means of protecting the organism from their possible toxicity.

TABLE 3
Relative potency of excitatory amino acids[a]

Group I	kainic acid
4+	quisqualic acid
	domoic acid
	allokainic acid
	N-methyl-D-aspartic acid
	β-N-oxalyl-L-α, β-diaminopropionic acid
	ibotenic acid
	tricholomic acid
	D-homocysteic acid
	N-methyl-DL-aspartic acid
	DL-homocysteic acid
	N-iminomethyl-D-aspartic acid
	N-ethyl-D-aspartic acid
	N-ethyl-L-aspartic acid
	DL-2-amino-4-sulfine-n-butyric acid
Group II	L-cysteic acid
3+	L-2-amino-3-sulfine-propionic acid
	N-n-propyl-D-aspartic acid
	L-homocysteic acid
	L-glutamic acid
	L-aspartic acid
	N-methyl-DL-glutamic acid
	N-methyl-L-glutamic acid
	N-methyl-D-glutamic acid
	N-methyl-L-aspartic acid
Group III	DL-2 amino-5-sulfo-n-valeric acid
2+	N-N-dimethyl-DL-aspartic acid
	D-glutamic acid
	D-aspartic acid
	D-cysteic acid
	N-methyl-DL-cysteic acid
	N-methyl-DL-homocysteic acid
Group IV	N-N-dimethyl-D-aspartic acid
1+	N-iminomethyl-L-aspartic acid
	N-ethyl-L-aspartic acid
	N-methyl-L-cysteic acid
	DL-α-amino adipic acid

[a] Relative potency of excitatory amino acids based on table from Reference 18 (used by permission of author and publisher). Additional data were taken from References 35, 37 and 38. Within each group, analogs are arranged in order of highest to least potent.

motor neurons (35, 37, 38, 104). A comparison of Table 1 with Table 3 illustrates that the order of neurotoxic potency of these amino acids parallels their neuroexcitatory potential. Thus, it was hypothesized by both Olney (77) and Johnston (35) that the neurotoxicity of the straight-chain and heterocyclic amino acids was an extension of their electrophysiological properties. It was further postulated that this interaction involved specific amino acid receptors that were present on the dendrites and soma but absent from the axons of various neurons, and resulted in profound alterations in membrane physiology causing cellular death (77).

There are several arguments against this hypothesis. First, glutamate is present in substantial quantities in the central nervous system and yet is quite harmless; and second, peripheral administration of glutamate does not increase glutamate levels in the CNS (62, 119). Subsequent studies, however, demonstrated that the membrane is well protected from extracellular glutamate by mechanisms for the active uptake, deamination and transamination of glutamate in both neuronal and glial elements, thereby maintaining extracellular glutamate concentrations at very low levels (21). Numerous attempts have been made to increase glutamate concentrations in the central nervous system by its peripheral administration, but none has been convincingly successful to date (62, 119). If, however, the blood-brain barrier admits monosodium glutamate to only a few restricted areas and if the concentration of glutamate in brain is relatively high, small increases in the overall glutamate concentration would not be detected even though concentrations of glutamate in the extracellular space might rise significantly in certain areas. Alone these lines, it has been suggested that the concentration of glutamate in the arcuate nucleus may increase as much as 4-fold following its systemic administration (89).

Support for this line of reasoning can be found in autoradiographic studies of the localization in brain of peripherally administered ^{14}C or ^{3}H-labelled glutamic acid (56, 68). Such studies suggest a clear correspondence between those areas demonstrating the greatest glutamate uptake and those areas that exhibit the greatest susceptibility to glutamate neurotoxicity. In addition, the autoradiographic localization appears to be largely on somal and dendritic processes of neurons.

The fact that the neurotoxic potential of

each of the straight-chain and heterocyclic glutamate analogs parallels its neuroexcitatory properties suggests that the neurotoxicity of these compounds might be a direct result of their ability to excite the membrane of the sensitive neuron (35, 77). For these reasons it is probable that all of the neuroexcitatory amino acids listed in Table 3 possess the potential to be neurotoxic. It is also possible that all of these neurotoxic compounds interact with similar but not necessarily identical receptor site(s) in or on the membrane, since their structural and functional relationships are quite similar. All possess a primary or secondary amino group *alpha* to a carboxylic acid that is 3 to 4 bond lengths removed from another acidic group such as a carboxyl, hydroxyl or resonance stabilized quinone (Table 4). These are the same structural requirements that Curtis and Watkins (19, 20) have suggested to be of importance for the neuroexcitatory properties of the straight-chain amino acids. Chemical modification of kainic or glutamic acid by the formation of diethyl or dimethyl ester derivatives, or the removal of the *alpha* carboxyl group inactivates both the excitatory and neurotoxic properties of these molecules (18, 20, 109). Parenthetically, it should be noted that *alpha* decarboxylation of the excitatory amino acids results in the corresponding GABA analogs which are potent neurodepressants (19, 20). The β- or γ-carboxyl or acidic group is not as important as a strong acidic group located *alpha* to the amino group, and apparently sulfinic or sulfonic acid residues as well as other minor acidic substitutes located β or γ to the amino group are sufficient to maintain the neurotoxicity of these compounds (77). The only exception to this rule appears to be cysteine, which induces a neurotoxic picture quite different than that seen following administration of the other neurotoxic amino acids (*vide supra*) (77, 79, 110). It has been suggested that the brain is readily permeable to cysteine, which is oxidized *in situ* to either cysteine sulfinic or sulfonic acid, resulting in a generalized neurotoxicity wherever cysteine has penetrated the brain (77). Supporting this suggestion has been the finding of cysteine oxidase throughout the neonatal and adult rat brain (59).

Not only does blockade of the carboxyl groups of glutamate and kainate inactivate their neurotoxic and neuroexcitatory potential but N-acetylation will also reduce their neurotoxicity (109).[2] Thus, it appears that derivatization or destruction of the glutamic acid backbone of the straight-chain and heterocyclic amino acids results in both a loss of neurotoxicity and neuroexcitatory properties. Recently, it has been reported that reduction of kainic to dihydrokainic acid results in the loss of its neurotoxic potential, a result which would be in apparent conflict with the structural and functional relationships outlined above (37, 109). It is quite possible that the reduction of the isopropylene side chain could result in a change of the solubility or conformational characteristics of the kainic molecule, such that its pharmacologically active subgroups are not brought into close contact with the membrane receptor.

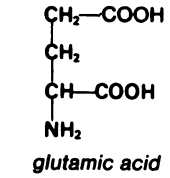
Another observation in apparent conflict with the supposition that the neurotoxic effects of these compounds are mediated through their neuroexcitatory properties is that glutamic acid diethylester does not appear to block the neurotoxic effects of kainate after intraocular or intrastriatal injection (109), although this glutamate derivative has been found to partially block the electroexcitatory effects of glutamate iontophoresed onto spinal motor neurons (61) or neurons in the CNS (31). It should be emphasized, however, that at the present there are as yet no highly active glutamate

[2] Aspartate appears to be an exception to the rule that derivatization of the amine interferes with its neurotoxic and neuroexcitatory effects, since N-methyl aspartate is one of the most potent neuroexcitant and neurotoxic amino acids, even more so than its parent compound, aspartic acid.

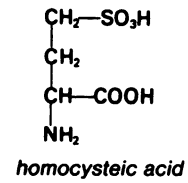
TABLE 4

Structure-function relationships of neurotoxic excitatory amino acids

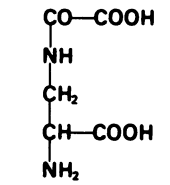
STRAIGHT CHAIN



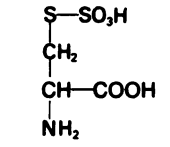
glutamic acid



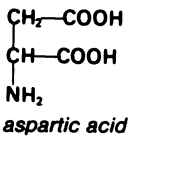
homocysteic acid



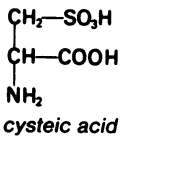
β-N-oxalyl-L-α, β-diamino-propionic acid



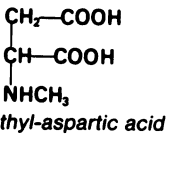
cysteine-S-sulfonic acid



aspartic acid

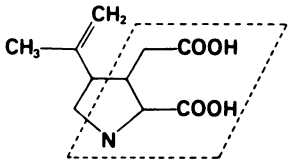


cysteic acid

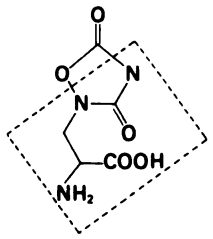


N-methyl-aspartic acid

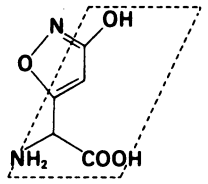
ISOXAZOLE-HETEROCYCLIC



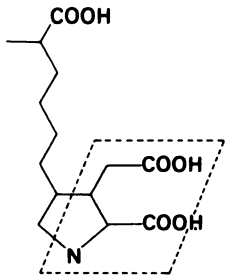
kainic acid



quisqualic acid



ibotenic acid



domoic acid

receptor-blocking agents and, although glutamic acid diethylester does appear to partially interfere with the interaction of glutamate at its receptor sites on spinal motor neurons and perhaps within the hippocampus, its effects do not appear to be profound (17, 31). Thus, it is difficult to conclude from current studies using these compounds that their failure to block the neurotoxic effects of kainate when injected into the eye or striatum can be taken as compelling evidence that the neuroexcitatory effects do not mediate the neurotoxic effects (36), especially in the absence of known dose-response relationships.

Neurophysiological effects of glutamic acid have been studied in several preparations. These studies suggest that superfusion of excitable neurons with glutamate results in a consistent and long-lasting depolarization of the cell, which persists for a considerable length of time after glutamate is removed from the superfusing medium (15, 39, 104). Similar results have been found with kainic acid but the depolarization induced by this amino acid is prolonged and at high doses may be irreversible (15) (one possible explanation for its greater neurotoxicity when compared with glutamate) (74, 82). Thus, it is possible that glutamate and its related neurotoxic analogs may alter the normal resting membrane potential of the target cell by interfering with sodium conductances in such a

way that the ability of the cell to maintain its ionic and osmotic gradients is lost and energy is futilely expended, leading to cellular death. Such an explanation is undoubtedly overly simplistic since, although both glutamate and dinitrophenol decrease the intraneuronal content of ATP to the same degree, dinitrophenol does not appear to reproduce glutamate neurotoxicity (51). It may well be that the pronounced ionic, osmotic and electrochemical alterations resulting from prolonged depolarization disrupt cellular regulatory mechanisms to the point that recovery is not possible. *In vitro* studies have also shown that both quisqualate and glutamate appear to open sodium channels in the locust muscle and frog spinal motor neurons (4, 15) and that the channel opening induced by quisqualate is more sustained than is that of glutamate (4), again providing a possible clue to the increased neurotoxicity and neuroexcitatory properties of some of the hetrocyclic glutamate analogs (35, 37, 38, 104, 109). Nevertheless, *in vitro* studies suggest that it is not sufficient to account for the neurotoxicity and neuroexcitatory properties of glutamate simply on the basis of a change in the sodium conductance of an excitable membrane, since tetrodotoxin blocks the initial excitatory depolarization but does not prevent the long-lasting depolarization induced by glutamate (15, 17). The long-lasting depression of the resting membrane potential of spinal motor neurons is reminiscent of the induction of cortical spreading depression by the local application of glutamic acid (9, 91, 120). Although the mechanisms for this latter event are not clearly understood, they are also not prevented by the direct application of textrodotoxin, suggesting that an action potential does not necessarily have to invade the area for the depolarization by MSG to be observed (30). The results of other electrophysiological experiments suggest that the membrane depolarization following the application of glutamate is characterized by a change in the potassium conductance, resulting in large extracellular potassium concentrations which further depolarize the cell and disrupt its ionic regulatory mechanisms (30, 122). Even these explanations are undoubtedly overly simplistic; alterations in membrane conductance for Na and K may be only contributing causes to the neurotoxic events mediated by the excitatory amino acids. Nevertheless, it appears that glutamate and its related analogs are toxic in high concentrations to all cells that possess glutamate-like receptors and relatively nontoxic to those compounds that do not possess these receptors. Thus, their neurotoxicity may result from a profound effect on membrane sodium and potassium conductances.

Recently, questions have been raised concerning such an interpretation of the events surrounding the neurotoxicity of glutamate and its heterocyclic analogs. Although it has been reported that kainic, ibotenic or quisqualic acid do not compete for the glutamate high affinity uptake site in neuronal membranes (6, 111), others have disputed this finding (45). Furthermore, it has been suggested by McGeer *et al.* (54), that removal of glutamate-containing corticostriatal afferents abolishes the neurotoxic effects of kainic acid injected directly into the striatum. These authors, however, did not use the same preparation to test the toxicity of systemically administered glutamate, a study which would have been of interest. Extracellular glutamate is normally disposed of by a high affinity presynaptic uptake (7, 21), characterized by uptake into glial cells, transamination into glutamine (7) and subsequent transferral into intraneuronal stores (7). Thus, it is conceivable that at least part of the neurotoxic effects of kainate and other glutamate-related analogs results from their interference with the normal disposal mechanisms for extracellular glutamate, and that their neurotoxicity is an indirect manifestation only of the neurotoxicity of glutamic acid. Compelling evidence against this postulate, however, comes from the studies of Coyle and Schwarcz, which suggest that conditions that are unfavorable

for the local release of other neurotransmitter systems do not block the neurotoxic effects of intraocular kainic acid (109). Furthermore, both in the eye and brain the toxicity of kainic acid, on a molar basis, is some 500-fold greater than that of glutamic acid. In view of the apparent lack of competition between glutamate and kainate for active transport into the cell (6, 111), it seems that a simple molar competition for high affinity uptake sites for glutamate cannot explain the neurotoxicity of kainic acid (74, 109).

Central to the idea that the neurotoxic effects of glutamate-related amino acids are a direct result of their excitatory potential has been the concept that both the excitatory and toxic events result from stimulation by these compounds of a single "glutamate-like" receptor. Lack of parallelism between the electrophysiological effects of glutamate and its related analogs would argue against this postulate. It has been shown that glutamate will stimulate the crayfish opener muscle, whereas kainate will not (110, 118). Furthermore, the excitatory effects of homocysteic acid on the frog motor neuron are blocked by chlorpromazine, diazepam and amitriptyline, whereas the excitatory effects of quisqualate are unchanged (25). Such data, however, do not prove that the neurotoxic effects of these compounds are not mediated through neuroexcitatory events, but merely suggest that there are more than one type of so-called "neuroexcitatory receptors" for the neurotoxic, neuroexcitatory compounds and that the affinity of these receptors for each amino acid may vary among species or areas, or both of the central nervous system. Heterogeneity of receptors for the amino acids, which although related are dissimilar, is suggested by the finding of two classes of receptors in cat spinal motor neurons; a population of "aspartate-preferring" receptors and another which is "glutamate preferring" (24, 55). Preference of these receptors for more rigid analogs such as N-methyl-D-aspartate on the one hand and kainate, on the other, is even more distinct (55). Aspartate-preferring receptors show a high affinity for the N-methyl-D-aspartate and the glutamate receptor, a greater affinity for the kainate. Therefore, it seems justified to conclude that there is a heterogeneous population of excitatory amino acid receptors without absolute specificity and that may be stimulated by high concentrations of amino acids for which they may possess a somewhat lower affinity. At present there are no known neurotoxic amino acids that are not capable of exciting those cells to which they are toxic. Thus, the most likely mechanism for the neurotoxic effects of these amino acids devolves upon their excitatory properties upon the target cell. Nevertheless, further confirmatory studies should be performed in order to verify such a postulate. Of particular interest would be the finding of related excitatory amino acids that are not neurotoxic, or vice versa.

VII. Neurotoxic Amino Acids as Research Tools

An unexpected offshoot of the interest in the neurotoxic amino acids has been their recent development as neurobiological research tools. Because of their rather unique properties of destroying cell soma while sparing the surrounding neuropil, these compounds have been used as a means for the selective lesioning of neuronal perikarya. This provides a technological advantage over other lesioning techniques which are incapable of distinguishing between somal, axonal and glial elements. Thus, glutamic acid, kainic acid or other neurotoxic amino acids can be stereotactically injected into discrete areas of brain, and the resulting effects on central nervous system physiology can be studied. (The major limitation to the intracerebral injections of the more potent compounds, especially kainic acid, is that they possess such marked excitatory potential that the treated animal develops recurrent generalized seizures which are often fatal. Current attempts to modify these seizures by treatment with diphenylhydantoin and barbiturates have, in our hands,

been largely unrewarding.) Similarly, systemic administration of these compounds results in reproducible lesions of the circumventricular organs, especially in the arcuate nucleus of the hypothalamus and the retina, and can be used for study of the effect of removing neuronal cell populations from these areas. It should be emphasized that one cannot assume the total destruction of all cell types following the injection of a particular neurotoxic amino acid into a given area of brain. Such caution is essential in view of the demonstration of resistant granule cells in the cerebellum (16, 29) and the probable heterogeneity of amino acid receptors throughout the CNS. Thus, histological conformation of all lesions should be considered essential. It may be possible in the future to make use of any heterogeneity of CNS amino acid receptors by developing specific antagonists or more selective neurotoxic receptor agonists in order to selectively lesion a specific set of neurons. It also seems probable, however (*vide supra*), that the injection of large amounts of the neurotoxic amino acids might be expected to override any receptor selectivity based on differing affinities and result in a generalized neurotoxic effect, provided the target cells possess at least one of the receptor types.

The potential usefulness of glutamic acid and its analogs in studying the physiology of the CNS is readily apparent from studies that have used peripherally administered glutamate (40) or a direct intraocular injection of kainate (106, 109) to study the cellular location of several different neurotransmitters and related synthetic enzymes in the retina. These studies, in conjunction with the histological evidence of a destruction of the inner layer of the retina, indicate that choline acetyltransferase, dopa decarboxylase, γ-amino butyric acid, glutamic acid, aspartic acid, alanine, glycine, tyrosine hydroxylase and glutamic acid decarboxylase are localized in the amacrine, bipolar and ganglion cells of the retina (13, 40, 51, 70, 106, 109). Furthermore, there is no apparent loss of adenylate cyclase in these preparations, indicating that the destroyed cells do not possess large amounts of this enzyme (106). After the intraocular administration of kainate, however, dopamine no longer activates the residual adenylate cyclase, suggesting that the destroyed cells are in some way responsible for mediating this response (106).

Similarily, direct intrastriatal injections of kainic acid have been made to study the normal physiological events relating to neurotransmitter control in this complex area (23, 108). Localized injections of kainate in the striatum markedly deplete glutamic acid decarboxylase and choline acetyltransferase while levels of tyrosine hydroxylase appear to be unaltered or increased (23, 108). These results suggest that the intrinsic neurons of the striatum are gabaminergic and cholinergic, and that the destruction of these cells results in a chronic increase in the turnover rate of dopamine in the unaffected dopaminergic presynaptic endings that arise from projections of substantia nigra. Furthermore, in a kainate-treated striatum, adenylate cyclase levels are reduced by 50% and the ability of dopaminergic agonists to activate this enzyme is also lost, suggesting a postsynaptic location for the dopamine responsive adenylate cyclase (16, 22, 23, 53, 107, 108). Of further interest is that apomorphine and haloperidol are still capable of increasing and decreasing rates of turnover of dopamine in the remaining dopaminergic terminals, providing what appears to be direct evidence for presynaptic regulatory dopaminergic receptors where effects are not mediated through adenylate cyclase (23). It has also been suggested that receptors for serotonin and acetylcholine are diminished (105, 107) whereas those for γ-aminobutyric acid are increased (107). These biochemical changes following injection of kainic acid into the striatum of rats are remarkably similar to the biochemical findings in the striatum of patients having died with Huntington's chorea, and have prompted several workers (108, 110) to propose that the kainate-injected rat striatum would be a suitable an-

imal model for the study of Huntington's chorea. Whether the assumption that the kainate-injected rat striatum is a representative model for the study of Huntington's chorea or is overly simplistic, awaits further study. Of interest is a recent finding (105) that portions of the postsynaptic membrane are still present in the kainate-treated striatum, thereby raising important questions as to the validity of a study of high affinity binding sites in this preparation.

Recently, it has been suggested from a study of the effect of kainic acid on aggregating fetal neuronal cells that the toxic effects of the neurotoxic amino acids may be specific only for gabaminergic and cholinergic cells and that catecholaminergic neurons may be relatively insensitive (34). Such a postulate from *in vitro* work contrasts with the finding of a reproducible toxicity of glutamate for dopaminergic cells in the hypothalamus *in vivo* (66, 69). The latter study clearly indicates that catecholaminergic neurons as a class are not uniquely insensitive to the neurotoxic amino acids.

The intracerebral injection of neurotoxic amino acids has also found use in the study of feeding and sexual behaviors. Localized injection of glutamate or kainate into the anterior lateral hypothalamus interferes with both these behaviors, suggesting that somal elements in this area are of critical importance (112).

Another use for the neurotoxic amino acids has been found in neuroendocrinology, where use has been made of the rather restricted hypothalamic neurotoxicity of parenterally administered monosodium glutamate to study the role of arcuate nucleus perikarya in the central regulation of endocrine function. As described previously, animals that have received moderate doses of monosodium glutamate during their neonatal life manifest rather bizarre behavioral, endocrinological and metabolic effects as adults (32, 66, 67, 69, 96, 117). These animals are stunted and obese with an increase in carcass fat and possess atrophic peripheral endocrine organs (66, 67, 69, 96, 110, 117). Studies have suggested that the peripheral hypogonadism of these animals is secondary to decreased hypothalamic release of LHRH (67). These animals are also hypothyroid despite apparently normal levels of serum TSH (67). They also possess elevated prolactin and decreased growth hormone levels (67). It appears certain that a central nervous system lesion is responsible for these diffuse neuroendocrine abnormalities in view of the fact that the pituitary response to LHRH and TRH is normal (67). Of great interest is the finding that the MSG-treated animal, despite his blindness and gross optic atrophy (13), still possesses a normal diurnal rhythm of pineal N-acetyltransferase, indicating that the retinal photoreceptors responsible for light-entrained rhythms are intact (67). Thus, the MSG-treated animal is an excellent model for separating visual cells from the photoreceptor cells responsible for biorhythms. The basal hypothalamus of the MSG-treated animal contains normal amounts of somatostatin (67), TRH (67) and LHRH (47, 67), providing further evidence that the perikarya lying within the arcuate nucleus do not supply releasing factors to the medium eminence. In addition, after castration of the MSG-treated animal, there is a normal postcastration rise in serum gonadotropin levels, indicating that the arcuate nucleus cells are not of primary importance in the regulation of this event (27). After administration of MSG neonatally, concentrations of glutamic acid decarboxylase, norepinephrine and serotonin within the medial basal hypothalamus of the adult appear to be normal. The only significant biochemical findings of note are reduced levels of dopamine and choline acetyltransferase in the arcuate nucleus and median eminence (67). These data have been corroborated by histological methods (11, 33, 60, 66).

The MSG-treated animal has also been used to delineate the presence of an arcuate nucleus-median eminence cholinergic system which had previously been unsus-

pected (11). Of further interest is the finding that the acute administration of MSG to adult rats results in qualitative abnormalities in serum, prolactin and growth hormone similar to those in the adult animal that had received MSG as a neonate (64). Histological damage to the arcuate nucleus does not occur, however, indicating that monosodium glutamate is not without significant pharmacological effects on endocrine regulation in the adult animal in the absence of neurotoxicity. It is clear that peripheral administration of MSG provides an excellent model to study basic neuroendocrine physiology of the arcuate nucleus.

There are other possible applications for the neurotoxic amino acids in neurological research and the relevance of such studies to human disease seems great. For example, adult animals that have received monosodium glutamate as neonates might serve as useful animal models of the Lesch-Nyhan syndrome (41). Like its human counterpart, the MSG-treated animal exhibits automutilation, and studies of this model might shed light on this peculiar behavioral anomaly of the Lesch-Nyhan-affected child.

Finally, one other area of possible neurobiological inquiry is suggested by the widespread distribution of the neurotoxic excitatory amino acids in the plant kingdom, in algae, mushrooms and other plants such as the chickpea. It is intriguing to speculate on the possible role that these neuroexcitatory neurotoxic amino acids play in the normal environmental interactions between such plants and cohabitating animal species.

VIII. Medical Implications for the Study of Neuroexcitatory Amino Acids

Research into the mechanisms for the neurotoxicity of the so-called neuroexcitatory amino acids may provide some rather important medical dividends. It is becoming quite clear that many of these compounds have a widespread distribution in the plant kingdom and their possible ecological importance for mammalian species is of great interest. Furthermore, it is conceivable that the elucidation of the mechanism of the neuronal destruction by compounds such as cysteine-S-sulfate and homocysteine might help further the development of receptor-blocking agents which might prevent some of the severe neuronal damage that appears to be a concomitant of such rare diverse diseases as sulfite oxidase deficiency and homocystinuria (41, 72, 80). Such a possibility is not overly speculative, since in the latter disease CNS deficits of glutathione do not appear to be the basis for its neuropathology (26, 90). It may be that as bizarre a disease as endemic neurolathyrism is related to the ingestion by susceptible individuals of a neurotoxic amino acid (β-N-oxalyl-L-α,β-diaminopropionic acid), found in the chickpea (12, 94, 95, 126).

It must be clearly stated that there is yet no proof that the neurotoxic amino acids are toxic after oral administration to primates. Experimental data in the rhesus monkey have been unconvincing (1, 2, 101, 116), and human children ranging in age from 3 to 10 have received as much as 20 to 36 g of monosodium glutamate per day for prolonged periods without any apparent lasting effect (58, 93, 127). Furthermore, glutamate does not appear to cross the placenta or appear in breast milk (114, 115). Nevertheless, it should be cautioned that in the human studies, specific deficits of the type which appear to exist in rodents were not specifically sought out and conceivably could be overlooked, especially in children already mentally retarded. One cannot state with certainty that changes in both intellectual capacity and reproductive function and normal endocrine regulation will not result from the ingestion of neurotoxic amino acids in large doses by *neonatal* humans. It is not sufficient to argue that the Chinese have used extracts of seaweeds for at least 1000 years with no apparent adverse cultural effects, because it has only been since 1910 that the industrial production of monosodium glutamate has made it possible to add MSG to foods in great quantity. As an anecdote, it is interesting to note that the first description of the Chinese restaurant syndrome was made by a

Chinese physician after arriving in the United States (44). Therefore, because there is no intrinsic value to the addition of monosodium glutamate to food as a flavor enhancer, it seems that the U. S. Food and Drug Administration acted wisely to remove this compound from baby food. It is not quite so clear what should be the policy of choice to follow in other circumstances involving neurotoxic amino acids. Kainic acid is used extensively as an antihelmintic in children in Southeast Asia and Asia Proper, and, although no specific neurotoxic effects have been observed, such a failure does not rule out the possibility of minor subtle alterations in either behavior or endocrine function of clinically important proportions. Perhaps the wisest course of action to follow would be to use such drugs only when absolutely necessary, and to maintain an active and close followup of those who do receive the drugs in an attempt to define clinically relevant syndromes.

Finally, much has been written concerning the so-called Chinese restaurant syndrome which occurs, repeatedly, in some people within a few minutes of partaking of Chinese food. The syndrome is characterized usually by flushing and a faint feeling, pain in the neck and other vasomotor phenomena that have been related to an acute reaction to monosodium glutamate used as a flavor enhancer in Chinese cooking. Some studies have failed to show a statistically significant production of any toxic side effects in controlled studies (100), whereas others have suggested that there is only a minor fraction of the normal population susceptible to monosodium glutamate but whose sensitivity is reproducible and predictable (42, 61, 98, 102). It seems likely that monosodium glutamate is partially or wholly responsible for the Chinese restaurant syndrome in these people and, if present data are confirmed, might serve as the first indication of a generalized neuropharmacological effect of glutamate in the human and as a further inducement to the study of neurotoxic amino acids in primates. It should also be added there are case reports of severe reactions in children to the ingestion of large amounts of MSG (97, 98).

IX. Conclusions

In conclusion, it is clear that MSG and its related straight-chain and heterocyclic analogs are neurotoxic for rodents and some fowl and appear to possess the peculiar property of interacting with membrane receptors on dendritic and somal elements of neurons to induce their destruction and necrosis attended by profound membrane depolarization. The peculiar properties of these compounds do not appear to be secondary to their active metabolism or uptake, but are probably due to a specific membrane effect which may partially depend on acute alterations in sodium and potassium conductances. It should be reiterated that the toxicity of monosodium glutamate and its analogs for primates has not been proven but it seems only prudent to avoid these compounds in clinical situations unless medically demanded.

Finally, the greatest potential for the neurotoxic amino acids is their use as biological research tools in the study of neuronal physiology within the CNS. These compounds may help to generate useful animal models of such diverse diseases as Huntington's chorea, Lesch-Nyhan syndrome, sulfite oxidase deficiency and perhaps homocystinuria.

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