Carbamate Formation and the Neurotoxicity of L-α Amino Acids

J. W. Olney et al. report (1) that the neurotoxicity of l-cysteine both in vivo and in vitro is mediated by the N-methyl-l-aspartate subtype of glutamate receptors and that in vitro physiological concentrations of bicarbonate potentiate this toxicity. Similarly, L-α-amino-β-methylaminopropionic acid (l-MeDAP, or β-N-methylamino-l-alanine, BMAA) has a low neurotoxicity in tissue culture in the absence of bicarbonate (2, 3). Similar effects have been observed with other basic amino acids (3). The interaction of bicarbonate with l-MeDAP in vitro leads to the formation of the α-carbamate, which has been established by 15N, 13C, and 1H magnetic resonance (NMR) spectroscopies (4).

We have confirmed (4) that interaction of bicarbonate with l-MeDAP (pH 7.5 to 8.5) leads to the formation of new species, which are readily observed in the 1H NMR spectrum and confirmed as carbamates by 13C NMR spectroscopy. One important feature of the 1H spectra is a quartet at δ = 4.2 ppm, the intensity of which increases with the addition of bicarbonate. This resonance is associated with the α-proton of the l-MeDAP adduct. The chemical shift is typical of that observed for an amino acid–CH–CH3 adjacent to an acetylated α-amino function (5, 6) and is pH-independent. Consequently we have proposed (3) that the initial reaction of l-MeDAP (1 to 2 hours) in bicarbonate-containing solutions leads to the formation of α-amino carbamate (Fig. 1). In the 13C NMR spectrum a new resonance in the carboxylate region at δ 179 ppm is observed.

The second pKₐ of cysteine (associated with the amine function) is 8.37; consequently a proportion of cysteine will be deprotonated at physiological pH and able to form an α-carbamate by reacting with the physiological carbonate buffer system (7).

![Fig. 1. Stereochemical similarities between the α-carbamate of an L-α amino acid (A) and NMDA (B), a consequence of their opposite chiralities.](image)

We have confirmed, by 1H and 13C NMR spectroscopy, that a new species is formed on the addition of bicarbonate to solutions containing cysteine (for example, at pH = 8.5, [l-cysteine] = 50 mM, [NaHCO₃] = 250 mM). The new signals (1H δ 4.18 ppm and 13C δ 181 ppm) are analogous to those observed for l-MeDAP and the changes are fully reversible on the addition of acid. Consequently the formation of an α-carbamate is suggested.

There are important stereochemical sequelae to these observations. First, the α-carbamates and free amino acids exist in equilibrium; chronic and acute toxicity may be the result of either or both of these molecules. Second, there is a striking structural resemblance between the α-carbamate of an L-α-amino acid (Fig. 1A) and NMDA (Fig. 1B). This stereochemical similarity is a consequence of the opposite chiralities of these molecules. The activation of NMDA receptors by α-carbamates formed by L-α-amino acids may be an important mechanism in the mode of action of endogenous and exogenous neurotoxic amino acids. Amino acids able to form carbamates of the kind in (Fig. 1A) at physiologic values of pH are all potential analogs of NMDA or other amino acids. Toxicity mediated by α-carbamates is a possible mechanism of chronic neurotoxic degradation in man.

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REFERENCES

8. September 1990; accepted 18 January 1991

Response: P. B. Nunn et al. provide important findings which, together with other evidence they cite, help to establish l-cysteine and l-MeDAP (abbreviated BMAA in other recent literature) as potentially important pathogenic agents in human neurodegenerative disorders. There are several intriguing parallels between l-MeDAP and l-cysteine. Both lack the α acidic terminal that other straight-chain excitotoxins possess, which may facilitate their entry into the brain. Previous studies (1, 2) have suggested that both l-MeDAP and l-cysteine, after entering the central nervous system, may be transformed by bicarbonate into unidentified molecules with more potent excitotoxic activity. The findings of Nunn et al. help clarify the nature of the bicarbonate effect—both molecules may be transformed into an α-aminocarbamate having properties similar to the powerful prototypic excitotoxin, NMDA. This suggests that there may be other α amino acids, either in the environment or brain, that appear quite innocuous but, under certain circumstances, can assume destructive properties similar to those of NMDA.

Other intriguing parallels pertain to the ability of both l-MeDAP and l-cysteine to injure or destroy neurons in the in vivo brain of experimental animals (2, 3), and evidence linking both molecules to three human neurological disorders—amyotrophic lateral sclerosis (ALS), parkinsonism, and Alzheimer's dementia. Heafield et al. (4) have described an apparent defect in cysteine catabolism that causes abnormally high cysteine-to-sulfate ratios in the blood of patients with these diseases. Each of these diseases occurs independently in sporadic distribution worldwide, but is also a component of a disease triad endemic to certain Pacific islands, especially Guam, where l-MeDAP is prevalent in the environment. It is difficult to explain this Guamanian syndrome solely in terms of an environmental factor because some victims, born on Guam, first experienced symptoms many years later after having left the island.

To explain all features of this syndrome, we suggest that Guamanians may have an inbred metabolic defect that causes elevated cysteine-to-sulfate ratios in the blood and chronically elevated cysteine concentrations in the brain. The presence of both cysteine and l-MeDAP in the Guamanian environment might accelerate onset of the disease among native inhabitants. People moving to other parts of the world would escape exposure to l-MeDAP but might, after a long latency, contract the disease, having taken with them the cysteine metabolic defect and being exposed to cysteine elsewhere in the world. The fact that cysteine is a more
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Science 251 (5001), 1619-1620.
DOI: 10.1126/science.1859531