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 nuclear 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 15N 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–CH2 NH2 CH3 adjacent to an acylated 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 an α-carbamate (Fig. 1). In the 13C NMR spectrum a new resonance in the carbonate region at δ 179 ppm is observed.

The second pK 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).

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 pD = 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 physiological values of pH are all potential analogs of NMDA or other amino acids. Toxicity mediated by α-carbamates is a possible mechanism of chronic neurological degradation in man.

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REFERENCES


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