Neurotoxic Potential of Three Structural Analogs of \( \beta\text{-N-oxalyl-}\alpha\text{-,}\beta\text{-Diaminopropanoic Acid (}\beta\text{-ODAP}) \)

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Lathyrism is a non-progressive motor neuron disease produced by consumption of the excitatory amino acid, 3-N-oxalyl-L-2,3-diaminopropanoic acid (\(\beta\text{-ODAP})\). To learn more about the mechanisms underlying Lathyrism three structural analogs of \(\beta\text{-ODAP}\) were synthesized. Carboxymethyl-A,B-diaminopropanoic acid (CMDAP) evoked inward currents which were antagonized by APV (30 \(\mu\text{M}\)), but not by CNQX (10 \(\mu\text{M}\)). N-acetyl-\(\alpha\text{-,}\beta\text{-diaminopropanoic acid (ADAP)}\) evoked no detectable ionic currents but potentiated N-methyl-D-aspartate (NMDA)-activated currents. The potentiation of NMDA currents by ADAP was blocked by 7-chlorokynurenic acid. Carboxymethylcysteine (CMC) did not activate any detectable ionic currents. None of the three \(\beta\text{-ODAP}\) analogs produced visible symptoms of toxicity in day old chicks when administered for 2–3 consecutive days. Ligand binding studies demonstrated that all the three compounds were effective in displacing \(^{3}\text{H}\)glutamate. The maximum inhibition was 92% for CMDAP, 61% for ADAP, 65% for CMC and 99% for \(\beta\text{-ODAP}\). These data indicate that analogs of \(\beta\text{-ODAP}\) may interact with glutamate receptors without producing neurotoxicity.

KEY WORDS: Excitotoxicity; Lathyrism; NMDA receptor; strychnine-insensitive glycine receptor; \(\beta\text{-N-oxalyl-}\alpha\text{-,}\beta\text{-diaminopropanoic acid; glutamate.}

INTRODUCTION

Lathyrism is a non-progressive motor neuron disease caused by the prolonged consumption of \textit{Lathyrus sativus} seeds containing the excitatory amino acid, 3-N-oxalyl-L-2,3-diaminopropanoic acid (\(\beta\text{-ODAP}), also called \(\beta\text{-N-oxalylamino-L-alanine or BOAA (1–3).}

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Throughout history outbreaks of the disease have occurred in many parts of the world; however, today the disease is mainly confined to north central India, Ethiopia and Bangladesh (2,4–6). Clinically, Lathyrism presents as a spastic paraparesis of the lower limbs resulting from degeneration of the corticospinal tract which originates from the pyramidal cells of the motor cortex. The disease is largely stable after consumption of the \textit{Lathyrus} seed ceases but, in some patients, there is a slow continual progression of clinical deficits with aging (7,8). The clinical and electrophysiological signs of the early reversible phase of the disorder have been reproduced in cynomolgus monkeys fed \textit{Lathyrus sativus (LS)}, LS extract with \(\beta\text{-ODAP}, or \(\beta\text{-ODAP}\) alone (3,9).

\(\beta\text{-ODAP}\) is an excitatory amino acid, which is neurotoxic to day old chicks, produces convulsions in...
mice and excitotoxic damage to mouse cortical neurons (10–12). Antagonists for the non-NMDA type excitatory amino acid receptor block acute neurotoxic responses in rodents (10,13). Ligand displacement studies and current-clamp observations suggested that β-ODAP binds with high-affinity to the AMPA-type glutamate receptor and with a lower affinity to the kainate receptor (14–16). Further, β-ODAP has stereospecific excitotoxic properties in rodents and in cortical and spinal cord tissue cultures (17–19). Quisqualate, like β-ODAP, is an agonist at AMPA-type receptors and may be a chronic neurotoxin in part by being taken up into presynaptic terminals, then slowly released. These data demonstrate that β-ODAP is an agonist at AMPA-type glutamate receptors in the central nervous system and may produce neurodegeneration by an excitotoxic mechanism. However, whether such a mechanism can explain lathyrism is not clear since despite the universality of AMPA receptors, there are extreme species differences in susceptibility to β-ODAP (12,21,22). Moreover the excitotoxic theory fails to explain why either the Swiss mice or albino rats are resistant to β-ODAP, while the day-old chicks or black mice are susceptible (12,23). Further recent studies have shown that β-ODAP is an inhibitor of tyrosine aminotransferase (TAT), a crucial enzyme involved in the metabolism of tyrosine and that this non-excitotoxic interaction might be the real mechanism β-ODAP neurotoxicity (23). Thus in order to examine further whether the excitotoxic potential that can be demonstrated for a compound in in vitro systems is in fact related to its neurotoxicity, three compounds structurally related to β-ODAP have been synthesized and their toxicological, neuropharmacological and electrophysiological properties have been compared.

**EXPERIMENTAL PROCEDURE**

**Synthesis of β-ODAP Analogs.** CMC and CMDAP were prepared by carboxymethylation of cysteine and diaminopropanoic acid (DAP) respectively, with monochloroacetic acid [Fig. 1]. The products were separated from the reaction mixture by chromatography on a Dowex-50-H’ column, followed by elution with water. The derivatives were recovered from the eluates by precipitation with aceton following concentration of the eluates to a small volume. ADAP was synthesized by reacting one equivalent of DAP-HCl under alkaline conditions with one equivalent of acetic anhydride under Schotten-Bouman conditions. ADAP was isolated from the reaction mixture by chromatography on a Dowex-50-H’ column and eluted with 2N HCl. ADAP was recovered from the eluate following concentration to a small volume and precipitation with acetone. The homogeneity of all the three compounds was established by paper chromatography in several solvent systems and their purity was established by elemental analysis and were found to have the following melting points: CMC 164°C, ADAP 204°C and CMC 202°C.

**Acute Toxicity Studies.** The compounds were dissolved in 0.5 ml of distilled water and administered intraperitoneally (1 mg/g body weight) to 2-3 day-old male white leg horn chicks that were observed periodically over 4-6 hours as described previously (12). The compounds were also administered for 3-4 consecutive days and the chicks observed.

**Preparation of Synaptic Membranes.** Crude synaptic membranes were prepared from the P2 pellets of brain homogenates of 2 day old white male leg horn chicks by a slight modification of the procedure described by Ross et al. (18). The crude synaptosomal pellet (P2) was rehomogenized in 0.2 mM Tris-EDTA-HCl buffer (pH 7.2) and centrifuged at 8,000 g for 20 minutes. The supernatant and the buffy coat were mixed and again centrifuged at 48,000 g for 20 minutes. The resulting pellet was washed four times with buffer and stored at -20°C until needed. Prior to use the membranes were washed twice in buffer and suspended in Tris (50 mM)-EDTA (1 mM)-HCl buffer (pH 7.2).

**Ligand Binding Studies.** Ligand binding assays were carried out using a slight modification of the procedure described by Monaghan and Cotman (24). Membrane suspensions (100 mg protein/50 ml) in triplicate were incubated with 10 nM [3H]glutamate in Tris (50 mM)-EDTA (1 mM)-HCl buffer (pH 7.2) in a final volume of 0.1 ml for 15 minutes at 37°C in a shaking water bath. The binding assay was terminated by centrifugation at 10,000 g for 2 minutes in a Beckman microfuge and the supernatant was carefully aspirated. The pellets were superficially rinsed twice with 0.5 ml buffer and the pellet was dissolved overnight at room temperature in 5 ml of Beckman BTS-450 tissue solubilizer. The bottom of the tubes containing the solubilized pellet were cut and placed directly into 10 ml of Bray’s solution and counted in a Beckman 6000 SE liquid scintillation counter with an efficiency of 47% for tritium. Nonspecific binding was determined by preincubating the membranes in the presence of glutamate (1 mM) for 15 minutes prior to the addition of the labeled ligand. The effect of the analogs on the binding of [3H]glutamate was examined by preincubating the membranes with ADAP, CMDAP or CMC at a concentration of 1 μM - 1 mM for at least 15 minutes prior to the addition of [3H]glutamate. The IC50’s were estimated by least square fitting to a plot of the mean specific [3H]glutamate binding.

**Hippocampal Cell Culture.** Hippocampal neurons were co-cultured with astroglial monolayers using previously described methods (25,26). The hippocampal were removed from 1 to 2 day-old Sprague-Dawley rats, the tissue minced, and incubated with papain for 45 min. The tissue was dissociated by gentle trituration and plated onto poly-D-lysine coated glass coverslips. Astrocytes survive and are allowed to grow for 7 days. The procedure was repeated with the dissociated cells plated onto the astroglial cell layer resulting in survival and growth of neurons. The neurons were maintained at 37°C in a sterile, humidified atmosphere (95% air-5% CO2) and fed twice weekly with Eagle’s minimum essential medium supplemented with 10% fetal calf serum.

**Electrophysiology.** Coverslips containing attached neurons were placed in a recording chamber located on the stage of an inverted microscope equipped with phase-contrast optics. The chamber was perfused at approximately 1 ml/min with a (extracellular) solution, at pH 7.3 and 310 mOsm containing in mM: NaCl 140, KCl 2.8, CaCl2 1.0, and HEPES 10. Microelectrodes, pulled in two stages (Narishige) from thin-walled borosilicate glass (World Precision Instruments), had resistances of 5–7 MΩ when filled with a (intracellular) solution, at pH 7.3 and 310 mOsm, consisting