Interaction of Some Plant Heterocyclic $\beta$-Substituted Alamines with Rat Brain N-Methyl-D-aspartate (NMDA) Receptors 1

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The neuropharmacological actions of some plant heterocyclic $\beta$-substituted alamines on rat brain N-methyl-D-aspartate (NMDA) receptors were studied. Of the compounds tested, 3-N-oxalyl-1-2,3-diaminopropionic acid ($\beta$-ODAP), the catalytic agent of human neurotolerancy, exhibited an inhibitory activity on the NMDA receptor binding assay at a relatively high concentration (IC$_{50}$; 4.7 $\times$ 10$^{-5}$ M). The biochemical precursor of $\beta$-ODAP, $\beta$-(isoxazol-5-on-2-yl)-l-alanine (BIA) was inactive in this assay. These results suggest that $\beta$-ODAP, the neurotoxin of Lathyrus sativus, in addition to its excitatory action on $\alpha$-amino-3-hydroxy-5-methylisoxazole-4-propanoic acid (AMPA) receptors, also has neurotoxic potential through its action on NMDA receptors.

Key words NMDA receptor; $\beta$-(isoxazol-5-on-2-yl)-l-alanine; $\beta$-ODAP; plant neurotoxin; non-protein amino acid; neurotolerancy

In the mammalian central nervous system (CNS), the NMDA-subtype of glutamate receptors are believed to be involved in neuronal processes associated with memory/learning and ischemia-triggered neuronal death. Considerable research activity has focused on the detailed analysis of the molecular mechanisms of potentiation of NMDA receptors. 2–6 There are at least six recognition sites on NMDA receptors: (1) a transmitter recognition site where glutamate or other EAAs are bound, (2) a glycine-modulatory site, (3) a voltage dependent magnesium-binding site, (4) a zinc-binding site, (5) a cation channel where phenycyclidine and MK-801 bind, and (6) a polyamine modulatory site. In the above neuronal events, ligand-dependent calcium ion flux through the cation channel plays an important role in the signal transduction processes.

Heterocyclic $\beta$-substituted alamines such as $l$-quissqualic acid (QA) and $\beta$-(isoxazol-5-on-2-yl)-l-alanine (BIA) have been isolated from several plant sources. 7 Some of these amino acids exert toxic or physiological effects on organisms in which they do not normally occur. For example, QA present in the Quisqualis species is a neuroexcitatory amino acid in mammals and activates the metabotropic glutamate receptor as well as the AMPA or QA receptor that is a major subtype of the non-NMDA ionotropic glutamate receptors. 2 Another example of such an amino acid with physiological activity in other organisms is BIA, a major non-protein amino acid found in seedlings of Lens, Pisiun and most of the Lathyrus species, which exhibits antimycotic and alelochemical activity. 8,9 This latter compound was recently recognized as a precursor in the biosynthesis of the neurotoxin 3-N-oxalyl-L-2,3-diaminopropionic acid ($\beta$-ODAP), the causal agent of human neurotoleranny in Lathyrus sativus. 11,12 $\beta$-ODAP is chemically and pharmacologically related to glutamic acid and shares its property of forming multiple conformations in solution; it is also known as a non-NMDA agonist that selectively acts on the AMPA subtype of glutamate receptors. 14 As these compounds, BIA and $\beta$-ODAP, have excitotoxic potential, we have investigated their actions on rat brain NMDA receptors using radioligand binding assays. In this paper we describe the interaction with the NMDA receptor of some heterocyclic $\beta$-substituted alamines from plants, including BIA and $\beta$-ODAP. A possible relationship between such activity and the etiology of neurotoleranny is also discussed.

MATERIALS AND METHODS

Chemicals BIA, $\beta$-(pyrazol-1-yl)-l-alanine ($\beta$-PA), L-laminosine, QA, L-willardiine, L-isowillardiine, L-lathyrine and $\beta$-ODAP were obtained from plants as described previously, 7 and dissolved in glass-distilled water. [3H]MK-801 and [3H]CPP were purchased from DuPont NEN. MK-801 was obtained from Research Biochemicals Inc. All other chemicals were of highest reagent grade and were obtained from Wako Pure Chemicals Inc.

Assays Radioligand binding assays for [3H]MK-801 15 and [3H]CPP 16 were performed as described previously, using a crude synaptic membrane (CSM) preparation of rat cerebral cortex which was frozen, thawed and washed thoroughly. In the case of CPP binding, CSM was treated with 0.02% Triton X-100 to remove endogenous ligands. Results were expressed as percentages of the control binding obtained with 5 $\times$ 10$^{-5}$ M MK-801 in the case of [3H]MK-801 binding or with 1 mM glutamate in the case of [3H]CPP binding, and were expressed as means of 4—7 experiments.

RESULTS

Effects of $\beta$-Substituted Alamines on [3H]MK-801 Binding We investigated the effect of some heterocyclic $\beta$-substituted alamines from higher plants on rat brain NMDA receptors using 2 $\times$ 10$^{-5}$ M [3H]MK-801 that acts

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β-substituted alanines have only a weak interaction, if any, with the cation channel site of the NMDA receptor.

**Effects of β-Substituted Alanines and β-ODAP on [3H]CPP Binding** As shown in Fig. 1, β-ODAP and QA exhibited a dose-dependent inhibition of [3H]CPP binding. The IC<sub>50</sub> values of β-ODAP and QA were 4.7 × 10<sup>-5</sup> m and 2.1 × 10<sup>-5</sup> m, respectively, at a concentration of 1 × 10<sup>-5</sup> m [3H]CPP. As QA is known as an agonist of AMPA-type EAA receptors,<sup>2</sup> this relatively weak action at the NMDA-competitive site indicates its wide spectrum of action on EAA receptors including the NMDA receptor. It was also interesting that BIA, the biosynthetic precursor of β-ODAP in *L. sativus*, had no apparent action on this binding site, while β-ODAP itself showed marked activity. L-Mimosine, L-isowillardiine and L-lathyrine also exhibited no inhibition of [3H]CPP binding up to 10<sup>-3</sup> m (data not shown in Fig. 1) and this was also the case for β-PA.

**DISCUSSION**

Our results suggest that β-ODAP has neurotoxic potential through its action at a relatively high concentration on the CPP binding site of rat brain NMDA receptors. QA, another plant non-protein amino acid, has a similar action. Both compounds are known agonists of AMPA-type EAA receptors. Other heterocyclic β-substituted alanines from plants, including L-lathyrine and L-willardiine, weakly inhibit [3H]MK-801 binding to the cation channel site of the NMDA receptor. BIA, the biosynthetic precursor of β-ODAP, which is a weak agonist of non-NMDA type receptors,<sup>17</sup> does not bind to either of the NMDA receptor sites.

β-ODAP has been reported to be the main causative agent in the pathogenesis of human neurolymphism.<sup>18</sup> It has also been proposed that β-ODAP, due to its ability to chelate Zn<sup>2+</sup>-ions, may indirectly activate NMDA receptors.<sup>19</sup> The results of the present study suggest that β-ODAP can also directly bind to the transmitter recognition site of the NMDA receptor.

As far as structure–activity relationships are concerned, glutamate can be compared with β-substituted alanines where the carboxymethyl-moiety of glutamate is exchanged for a heterocyclic or an aliphatic group with one or more carbonyl-moieties, such as the isoxazolin-5-one ring in BIA or the oxalylamino-group in β-ODAP. Like glutamate, β-ODAP can have different conformations in solution,<sup>13</sup> and perhaps the different conformations bind to different sites at the NMDA or non-NMDA (AMPA or kainate) subtypes of glutamate receptors. These conformation–activity questions may perhaps be answered by NMR.

Ligand binding assays only show that the active substance is competing with radioligand for the same binding site, and may not necessarily reflect its neuropharmacological activity. Electrophysiological experiments, such as measurement of the cation conductance at the NMDA-gated channel, using recombinant cDNA techniques, should provide more detailed information about the neurological action of these excitatory amino acids. Such experiments are now being planned.
REFERENCES

1) Abbreviations: NMDA: N-methyl-D-aspartate; EAA: excitatory amino acid; AMPA: a-amino-3-hydroxy-5-methylisoxazole-4-propanoic acid; CPP: 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid.


