Glutamate Uptake System in the Presynaptic Vesicle: Glutamic Acid Analogs as Inhibitors and Alternate Substrates*

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A variety of naturally occurring amino acids, their isomers, and synthetic analogs were tested for their ability to inhibit uptake of [3H]glutamate into presynaptic vesicles from bovine cerebral cortex. Strongest inhibition (K<sub>i</sub> < 1mM) was observed for trans-l-aminocyclopentane-l,3-dicarboxylic acid (t-ACPD) and erythro-4-methyl-L-glutamic acid (MGlu), while 4-methylene-L-glutamic acid (MeGlu) was only moderately inhibitory (K<sub>i</sub> = ~3mM), indicating that the synaptic vesicle glutamate translocator has higher affinity for trans-ACPD and MGlu than for glutamate. A few other amino acids, e.g., 4-hydroxyglutamic acid, S-carboxyethyl cysteine, and 5-fluorotryptophan, were slightly inhibitory; all l- and dl-isomers of protein amino acids and longer chain acidic amino acids were without measurable inhibition. Potassium tetrahydronitrate and S-sulfocysteine exhibited strong to moderate noncompetitive or irreversible inhibition. Inhibition by t-ACPD, MGlu, or MeGlu was competitive with glutamic acid. Each of these competitive inhibitors was also taken up by the vesicle preparation in an ATP-dependent manner, as indicated by their being recovered unchanged from filtered vesicles. Similar results were obtained with reconstituted vesicles, while glutamate uptake by partially purified rat synaptosomes was inhibited only by MGlu. These results indicate that the glutamate translocator of presynaptic vesicles has stringent structural requirements distinct from those of the plasma membrane translocator and the metabotropic type of postsynaptic glutamate receptor. They further suggest possible structural requirements of pharmacologically significant compounds that can substitute for glutamic acid in the presynaptic side of glutamatergic synapses, thus serving to moderate or control glutamate excitation and associated excitotoxic effects in these neurons.

KEY WORDS: Synaptic vesicles; glutamate; ACPD; neurotransmitters.

INTRODUCTION

It is now widely accepted that glutamate functions as a major excitatory neurotransmitter in the vertebrate CNS and that it is involved in memory and learning and in a variety of pathophysologies in the brain (for reviews, ref. 1-2). There is increasing evidence that synaptic vesicles play a role in this process (3-4). Ca<sup>2+</sup>-dependent release of glutamate from a non-cytosolic pool (5,6) and ATP-dependent uptake of glutamate into highly purified, isolated synaptic vesicles (7-10) have been shown to occur. The vesicular glutamate uptake is driven by an electrochemical proton gradient that is generated by a proton-pump Mg-ATPase, and stimulated by physiologically relevant concentrations of chloride (8-11,30). The key position of the vesicular uptake system in determin-
ing the function of glutamate as a neurotransmitter suggests an interest in pharmacological means by which vesicular uptake can be modulated. Previous studies reported that a few close analogs of glutamate inhibited its uptake, apparently by acting as a competitive substrate (8); however, strongest competitive inhibition was observed by the peptide-containing ergot bromocriptine (12), although the competitive nature of this inhibition has recently been questioned (13). Recently, sulfur amino acids analogous to glutamate have also been reported to be inhibitors (14). In this communication, we further examine the specificity of the glutamate uptake system, and demonstrate for the first time that certain close analogs of glutamate are similarly transported, while other apparently inhibitory compounds probably act at other sites, possibly in the Mg-ATPase proton pump.

**EXPERIMENTAL PROCEDURE**

Purified bovine synaptic vesicles were prepared as previously described (15). The preparations typically had specific activities of glutamate uptake of 400-1000 pmol/10 min/mg protein when assayed with 0.05 mM glutamate. ATP-independent glutamate uptake was typically less than 10% of the ATP-dependent uptake in the absence of Na+ ion. Bovine synaptic vesicle preparations were solubilized and reconstituted according to the method of Carlson et al. (12); the reconstituted fraction had approximately twice the specific activity of the original vesicles. Synaptosomes were prepared from rat cerebral cortex by the method of Cotman (17).

Glutamate uptake was assayed in vesicle preparations essentially as described previously (8). Reaction tubes containing the test amino acid and vesicles were preincubated at 30°C for 5 min prior to addition of L-[G-3H]glutamate and ATP. Incubations were also carried out in the absence of ATP. After 1.5 min incubation, the reactions were stopped by addition of cold 0.15M KCl and filtered as usual. Filters were then shaken with 7 ml liquid scintillation cocktail for at least 1 hr and counted. Those values observed in the absence of ATP were subtracted from values observed for the complete system to obtain the ATP-dependent uptake activity. For measurement of uptake of unlabeled amino acids, the filters were shaken overnight with 80% ethanol in water, followed by centrifugation to remove the residue of the filter and washing of the residue. More than 95% of 3H-glutamate radioactivity taken up by vesicles was extracted from the filters by this procedure. The combined supernatant and wash was evaporated to dryness. For some experiments, this residue was redisolved in water and passed through a small (0.6 x 1.5 cm) column of Dowex-1 acetate. The column was washed with 4 ml water, eluted with 2 ml of 2N acetic acid, and the eluent evaporated to dryness. The Dowex-1-treated samples were cleaner and easier to filter after derivatization, but there was no difference in the chromatograms of the acidic amino acids. Dried residues from either treatment were derivatized with PITC 2, treated samples were cleaner and easier to filter after derivatization, and washing of the residue. More than 95% of 3H-glutamate radioactivity was taken up by vesicles. Synaptosomes were prepared from rat cerebral cortex by the method of Cotman (17).

Inhibition of [3H]-glutamate uptake into synaptic vesicles by various naturally occurring and synthetic amino acids is shown in Table I. At the lowest concentration used (2.5 mM), only the three acidic amino acids having the same charged groups in the same relative position as glutamate showed inhibition of 50% or more. When a lower but definite level of inhibition was observed, additional concentrations of the compound were tested; 5-fluorotryptophan and S-sulfocysteine showed concentration-dependent inhibition, although inhibition comparable to the substituted glutamates was seen only

2 Abbreviations used: PITC, phenylisothiocyanate; PTC, phenylthio-carbamyl; ACPD, 1-amino-cyclopentane-1,3-dicarboxylic acid (cis or trans DL); MeGlu, 4-methylglutamyl acid (racemate or unspecified optical isomers); MgGlu, 4-methylglutamyl acid (unspecified diastereois or optical isomers). and the PTC amino acid separated and quantitated by the Waters Pico-