EFFECT OF SOME "STRONG" EXCITANTS OF CENTRAL NEURONES ON THE UPTAKE OF L-GLUTAMATE AND L-ASPARTATE BY SYNAPTOSOMES

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SUMMARY: "Strong" excitants of central neurones such as β N-oxalyl L α,β -diaminopropionic acid (ODAP), N-methyl-D-aspartic acid (NMDA) and kainic acid (KA) were found to inhibit the high affinity uptake of glutamate and aspartate in synaptosomes isolated from young rat brain. The potency of these "strong" excitants as convulsants appear to parallel their ability to inhibit glutamate uptake by synaptosomes. The data suggest the possibility that the convulsive effect of these "strong" excitants could be mediated by glutamate/aspartate.

Studies in recent years have shown that amino acids such as glutamate and aspartate excite most central neurones and could function as neurotransmitters (1-3). Following the synaptic release of these amino acids, inactivation of the transmitter appears to involve a reuptake mechanism by an active transport process (4). In support of this, high affinity uptake systems have been identified for the transmitter amino acids in neural tissue (5-7)

In a recent study, Balcar and Johnston (8) have shown that "strong" excitant amino acids such as N-methyl-D-aspartic

acid (NMDA) and β-N-oxalyl-L-α,β-diaminopropionic acid (ODAP) do not inhibit the high affinity uptake of glutamate by rat brain slices and have concluded that the measured glutamate uptake and its ability to depolarize do not appear to be directly related. In a subsequent study with ODAP, we have found that it does inhibit glutamate uptake by synaptosomes isolated from young rat brain and adult monkey spinal cord(9). This prompted us to think that the effect seen with brain slices may not fully reflect the interaction at the level of the synaptic elements. We, therefore, reexamined the effects of NMDA, ODAP as well as kainic acid, a derivative of pyrrolidine-2-carboxylic acid (10) on L-glutamate and L-aspartate uptake by synaptosomes isolated from young rat brain.

MATERIALS AND METHODS: Synaptosomes were isolated from 12 day old rat (18-22 g) brain by the procedure of Eichberg *et al* (11) except that the P_2 pellet was isolated by centrifuging the P_1 supernatant at 7000 g for 30 min instead of 10,000 g for 60 min. This modification according to Levitan *et al* (12) results in a purer preparation of synaptosomes in the subsequent gradient.

(U-^{14}C)L-Glutamate (99 mCi/mmole) and (U-^{14}C)L-aspartate (80 mCi/mmole) were used with suitable additions of the

respective cold amino acid to make up the required concentration of amino acid in the incubation medium. The amino acid concentration range employed was from 10^{-4}M to 10^{-5}M representing essentially the high affinity substrate concentration used for isolated nerve endings (7,13). The incubation medium used was the same as described by Peterson and Raghupathy (14). The incubation mixture in 1 ml volume contained 10 mM Tris-HCl (pH 7.4), 15 mM $MgCl_2$, 150 mM NaCl and 0.25 µCi of the labeled amino acid. The "strong" excitants were added at a concentration of 10^{-3}M (pH 7.4). The reaction was started by the addition of synaptosomes (200 µg protein) and the incubation was carried out in 5 ml flasks with shaking at 37° for 3 min. At the end of the incubation period the contents of the flasks were chilled by the addition of ice cold incubation buffer and immediately filtered onto a nitrocellulose membrane filter (0.45 µ). The filter disc was washed with 30 volumes of cold buffer, dried and counted in a Beckman LS-100 scintillation counter using 10 ml of toluene containing 0.5% PPO (W/V). The maintainance of the integrity of synaptosomes under experimental conditions was ascertained by measuring the lactic dehydrogenase activity of the sample (15) as well as by demonstrating that practically no radioactivity was retained on the filter when the samples after incubation were lysed with water before filtration.

RESULTS AND DISCUSSION: Studies by different groups of workers have established high and low affinity uptake systems exist for transmitter amino acids in the neural tissues. These systems have been demonstrated with brain slices, homogenates, as well as isolated nerve endings (5,7,13). In the present investigation the effect of "strong" excitants such as ODAP NMDA and kainic acid was first studied on the synaptosomal uptake of glutamate at 10^{-5}M concentration in the medium. The results indicated that ODAP, NMDA and kainic acid inhibit glutamate uptake by 17%, 67% and 70% respectively. In brain slices Balcar and Johnston (8) have found that ODAP and NMDA at a concentration of 10^{-4}M have nosignificant effect on the uptake of glutamate present at a concentration of 10^{-8}M representing the high affinity uptake system in slices. Kainic acid has been found to be a weak competitive inhibitor of glutamate with a Ki of 250 µM (Johnston, personal communication). Fig. 1 gives the detailed effect of three "strong" excitants of central neurones on glutamate uptake by synaptosomes. It is clear that the effect of the "strong" excitants is felt over a range of glutamate concentration depicting the high affinity uptake region. Fig 2 gives essentially similar results for aspartate uptake except that NMDA appears to be a more potent inhibitor with glutamate than with aspartate. It has been shown that glutamate and

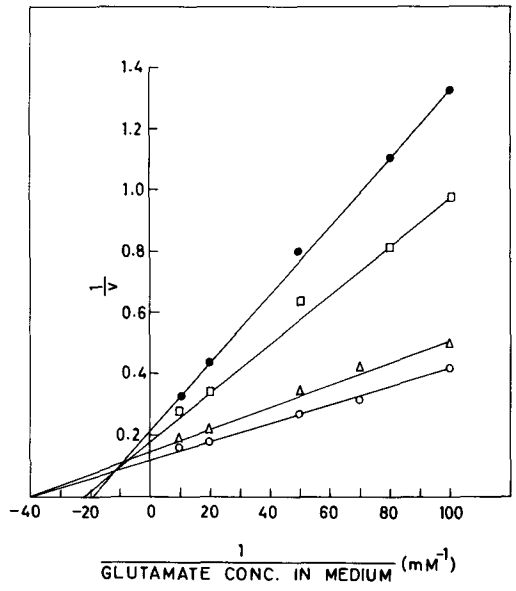

Fig. 1 Effect of ODAP, NMDA and kainic acid on the uptake of L-glutamate by synaptosomes isolated from young rat brain.

v is expressed as nmoles of glutamate taken up per mg protein per 3 min.

O - Control uptake; △- uptake in presence of 10^{-3}M ODAP; □ - uptake in presence of 10^{-3}M NMDA and ● - uptake in presence of 10^{-3}M kainic acid.

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aspartate are taken up by the same transport system (5,7) and the "strong" excitants would be expected to have identical effects on the uptake of the two dicarboxylic amino acids.

It appears that the lack of any significant effect of these "strong" excitant amino acids on glutamate uptake in

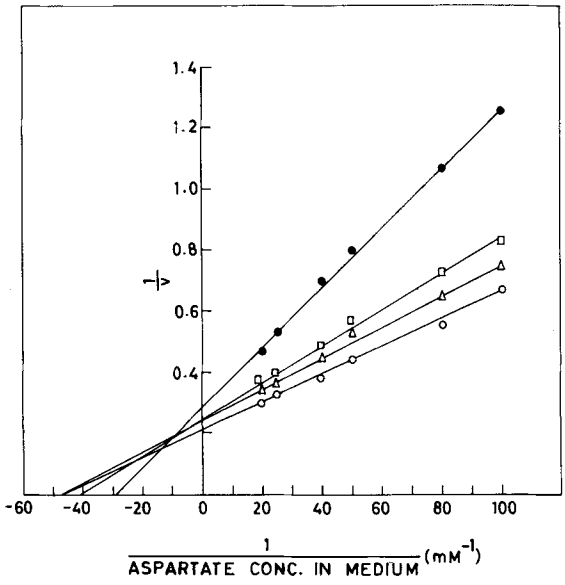

Fig. 2 Effect of ODAP, NMDA and kainic acid on the uptake of L-aspartate by synaptosomes isolated from young rat brain.

Units of v are as given in Fig. 1.

O - Control uptake; △ - uptake in presence of 10^{-3}M ODAP; □ - uptake in presence of 10^{-3}M NMDA and ● - uptake in presence of 10^{-3}M kainic acid.

brain slices, could be due to the competing effect of the various cell constituents for the inhibitor as well as the substrate. Even with isolated synaptosomes much significance can not be attached to the type of interaction observed. It appears that the "strong" excitants alter the Km as well as Vmax, with the effect on Km being generally more pronounced.

This interaction could as well be determined by the purity of the synaptosomal preparation as well as by the heterogeneity within the synaptosomal population since unique synaptosomal fractions manifesting high affinity uptake for glutamate and aspartate have also been recognised (16).

It is of importance to report that the"strong" excitants studied in the present investigation induce convulsions in young rats. With this experimental system Johnston (17) has observed that NMDA is more potent than ODAP. Kainic acid has been found to be more potent than NMDA. (Johnston, Personal communication). Thus, the potency of these "strong" excitants as convulsants appear to parallel their ability to inhibit glutamate uptake by synaptosomes. It is clear that these "strong" excitants do interfere with the high affinity transport processes for glutamate and aspartate. While interaction between the "strong" excitants and glutamate/aspartate at the level of post synaptic receptor, glial cell uptake and enzyme catalysed reactions could also prove to be important, the present results emphasize the possibility that the convulsive effect of these "strong" excitants could be mediated by glutamate/aspartate.

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