Life Sciences Vol. 22, pp. 2009-2016 Pergamon Press
Printed in the U.S.A.

# AMINO ACID TRANSPORT: ALTERATIONS DUE TO SYNAPTOSOMAL DEPOLARIZATION

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(Received in final form April 21, 1978)

## SUMMARY

The effect of a preliminary high potassium depolarization on the synaptosomal transport of a number of compounds was tested. It was found that a preliminary depolarization increased the transport of glutamic acid, aspartic acid, glycine and choline, all of which are known or suspected neurotransmitters or neurotransmitter precursors. Depolarization had no effect on the transport of a number of other compounds including the neurotransmitter precursor, tyrosine. The increased transports of glutamate, aspartate, glycine and choline were found to be into osmotically sensitive compartments and were not due to decreases in leakiness of synaptosomes. Kinetically, the increase in choline transport was characterized by an increase in $V_{max}$ and the increases in glutamate and glycine transport were characterized by decreases in $K_T$. Thus, selected transport systems in synaptosomal preparations can be altered by a previous depolarization.

It has been known for some time that synaptosomal fractions are endowed with transport systems for a variety of compounds, including amino acids, neurotransmitters and neurotransmitter precursors (1-7). Some of these transport systems are selectively localized to particular neuronal pathways in the brain (4) and have a high affinity for their substrate, i.e. a $K_T$ for transport in the micromolar range (8-15).

The high affinity transport system for choline has been studied extensively and there is evidence supporting the idea that it plays a regulatory role in the synthesis of ACh (16). One of the most interesting properties of this choline transport system is that its capacity appears coupled to neuronal activity. When an animal is subjected to various treatments which increase or decrease the activity of cholinergic neurons, one finds a parallel increase or decrease in high affinity choline uptake *in vitro* (17-20) and *in vivo* (21,22). The *in vivo* activation of choline uptake can be mimicked by *in vitro* depolarization (23,24). This finding led us to investigate whether transport systems in addition to that for choline could be activated by *in vitro* depolarization. We find evidence that several, but not all synaptosomal transport systems will respond to depolarization and perhaps also to changes in neuronal activity. A preliminary report of this work has appeared (25).

## METHODS AND MATERIALS

*Methods.* The experimental procedure has been described in detail previously (24) and is, briefly, as follows. Rat (male, Sprague-Dawley, 180-

0300-9653/78/0612-2009$02.00/0

220 g) cerebral cortex and spinal cord were homogenized in 20 volumes of 0.32 M sucrose. Spinal cord was used in some of the glycine studies because glycine is thought to have neurotransmitter function in the spinal cord but not in the cortex (8). Crude mitochondrial pellets ($P_2$) containing the synaptosomes were obtained by differential centrifugation. The pellets were re-suspended either in ice-cold Krebs Ringer phosphate (control medium, KRP: NaCl, 122mM; KCl, 4.9 mM; $CaCl_2$, 1.3 mM; $Na_2HPO_4$, 15.8 mM; $MgSO_4$, 1.2 mM; dextrose, 11.1 mM; pH 7.4 at 37° C) or in ice-cold 62 mM $K^+$-KRP (depolarizing medium: same as KRP except 62 mM KCl, 65 mM NaCl) and were incubated for 10 min at 37° C (preliminary incubation). In the following experiments, depolarization always took place in the preliminary incubation and never during measurement of uptake. The preliminary incubation media were then centrifuged and the synaptosomal pellets ($P_2$) were re-suspended in ice-cold 0.32 M sucrose. Aliquots of this suspension were used for uptake studies. While these suspensions are enriched in synaptosomes, they also contain mitochondria, myelin fractions and glial fragments (26). For some substances, such as choline, it appears that most of the transport is synaptosomal (15, 27) while for others glial fragments may participate in the transport (28).

Uptake consisted of a 5 min equilibrium period in KRP at 37° followed by addition of the radiolabeled compounds and an additional 4 min incubation at 37°, with the following exceptions. Preliminary experiments indicated that it was necessary to eliminate the 5 min equilibration period when measuring glycine transport in cortex and that glutamate and aspartate uptakes were linear for only 2 min. Uptakes were stopped by addition of ice-cold Krebs Ringer phosphate followed by centrifugation. Temperature-dependent uptakes were determined by subtracting uptake values for synaptosomes incubated under identical conditions at 4° C from uptake values at 37° C. Sodium-dependent uptakes were determined by subtracting uptake values for synaptosomes incubated at 37° C in sodium-free KRP (sucrose and Tris phosphate replace NaCl and $Na_2HPO_4$ respectively) from uptake values in normal KRP.

Statistical comparisons were made with student's two tailed t-test and $p < .05$ was assumed to be the minimal level for a significant difference. Kinetic parameters were determined by plotting V vs V/S and 1/V vs 1/S (29).

Materials. [Me-$^3$H]Choline, 10 Ci/mmole; [$^3$H]aspartic acid, 0.3 Ci/mmole; and [1-$^3$H]taurine, 15 Ci/mmole, were obtained from Amersham/Searle, Arlington Heights, IL. [3-$^3$H]Alanine, 16 Ci/mmole; [3-$^3$H]arginine, 15 Ci/mmole; [8-$^{14}$C]adenine, 58 mCi/mmole; [2,8-$^3$H]adenosine, 31 Ci/mmole; [2,3-$^3$H]GABA, 35 Ci/mmole; [3,4-$^3$H]glutamine, 33 Ci/mmole; [2,3-$^3$H]glutamic acid, 17.5 Ci/mmole; [2-$^3$H]glycine, 15 Ci/mmole; [4,5-$^3$H]leucine, 40 Ci/mmole; [3-$^3$H]-ornithine, 26 Ci/mmole; [$^3$H]serine, 5 Ci/mmole; [$^3$H]proline, 2.5 Ci/mmole; [ring 3,5-$^3$H]tyrosine, 40 Ci/mmole, were obtained from New England Nuclear Corp., Boston, MA.

## RESULTS

Effects of preliminary depolarization on synaptosomal transport. It was found that preliminary depolarization of synaptosomal fractions results in increased transport of several of the compounds tested. The uptakes of choline, aspartate, glutamate and glycine in cerebral cortex tissues and glycine in spinal cord tissues were significantly greater following a depolarizing preliminary incubation compared to a non-depolarizing or control preliminary incubation (Table 1). On the other hand, depolarization had no effect on the transport of a large number of compounds. These included adenine, adenosine, alanine, arginine, glutamine, leucine, ornithine, proline, serine, taurine and tyrosine.