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SELECTIVE EFFECTS OF LITHIUM ON SYNAPTOSOMAL AMINO ACID TRANSPORT SYSTEMS

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Amino acids regarded as putative neurotransmitters are transported into synaptosomes by high affinity, Na^+-dependent systems. Such transport systems have been demonstrated for γ-aminobutyric acid (GABA),[1] aspartic acid,[2,3] glutamic acid,[3] proline[2] and glycine.[4,5] The present communication describes a special effect of Li^+ on the synaptosomal accumulation of aspartic acid. It is shown that, in the absence of Na^+, Li^+ substantially supports the uptake of aspartic acid, and to a lesser extent that of glutamic acid and proline. In contrast, Li^+ cannot replace Na^+, to any extent, in the uptakes of GABA and glycine.

Synaptosomal fractions were isolated from brains of adult rats and assayed for transport activity as described elsewhere.[2] Synaptosomal protein (0.25 mg) was incubated with the labeled amino acid in 1 ml of a medium composed of Tris HCl (15 mM, pH 7.4), and either NaCl (175 mM, medium TNa), LiCl (175 mM, medium TLi) or sucrose (350 mM, medium TS). Incubations were carried out for 2 min at 37°.

Data from a representative experiment are given in Table 1. In agreement with previous reports, the accumulations of glutamic acid, aspartic acid and proline were nearly completely dependent upon the presence of Na^+ in the incubation medium. The relatively small accumulations of these amino acids occurring in medium TS are possibly mediated by low-affinity non-Na^+-dependent transport systems (see reference 6).

The accumulation of aspartic acid from medium TLi is ten times greater than that from medium TS, and is about one-third of the Na^+-dependent accumulation from medium TNa. The uptakes of proline and glutamic acid from TLi medium are significantly greater than that from medium TS, but comprise only a small fraction of the Na^+-dependent accumulation.

TABLE 1. Effects of Lithium on Amino Acid Accumulation by Synaptosomal Fractions

Amino acid*	Activity accumulated from medium† (cpm)		
	TS	TNa	TLi
Aspartic acid	1,220	43,000	12,200
Glutamic acid	680	31,900	1,780
Proline	117	2,550	390
GABA	285	14,600	240
Glycine‡	1,720	4,330	1,580

†TS is Tris-sucrose, TLi is Tris-lithium chloride and TNa is Tris-sodium chloride. Concentrations and incubation conditions are given in text.

‡Glycine uptake by synaptosomal fractions from brain stem tissue. The uptakes of the other amino acids were by fractions from cortical tissue.

*The specific activities of the amino acids are: ^{14}C-aspartic, 209 mCi/m-mole; ^{14}C-glutamic acid, 238 mCi/m-mole; ^{14}C-glycine, 104 mCi/m-mole; ^{14}C-proline, 250 mCi/m-mole; and ^{3}H-GABA, 10 Ci/m-mole.

Our previous studies[2] have indicated that the interaction of Na^+ with the aspartic acid transport system is second order with respect to the cation. The Hill plot of this activation was linear with an integral slope value of 2, indicating complete interaction of both sodium ions. A similar analysis of the activation of aspartic acid accumulation by Li^+ shows the Hill plot to be linear, but with a non-integral slope value of between 1 and 2 (the average of three determinations was 1.34 ± 0.08). This suggests that the interaction is second order with respect to Li^+, but that the second lithium ion may not be fully active (see reference 7).

In synaptosomal fractions from brain cortices of adult rats, glycine is accumulated about equally via a system which is totally dependent on Na^+ and a non-Na^+-dependent system.[4] In fractions prepared from brain stem, however,

glycine is transported predominantly by a Na^+-dependent process (Table 1); the requirement for Na^+ cannot be replaced to any extent by Li^+. The data in Table 1 also demonstrate the strict Na^+ requirement for synaptosomal uptake of GABA, and show that uptake of GABA from LiCl medium is no greater than that achieved in the absence of the cation.

Results of additional experiments not reported here indicate a number of complexities in the interaction of Li^+ with the transport systems. For example, the uptake of aspartic acid from LiCl medium, in proportion to its uptake from NaCl, appears to decrease with decreasing incubation temperatures, thereby suggesting that a higher energy barrier is associated with the Li^+-supported uptake. We have also noted that the ratio of Li^+/Na^+-supported uptake is slightly reduced if Mg^{2+} were included in the incubation medium. A detailed study of these and other aspects of Li^+-supported amino acid uptake will be necessary to assess the significance of this Li^+ effect vis-à-vis its pharmacological actions. Nevertheless, it is intriguing to note that the supportive effects of Li^+ occur only in the transport of the putative neurotransmitter amino acids which have been associated with excitatory properties (aspartic acid and glutamic acid). The uptakes of the two amino acids which have been identified as inhibitory, glycine and GABA, are not at all affected by Li^+ Although no data are available on the nature of the synaptic action of proline, an excitatory effect of this amino acid would be consistent with the pattern indicated by the data presented.

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REFERENCES

1. D.L. Martin and A.A. Smith III, J. Neurochem. 19, 841 (1972).

2. N.A. Peterson and E. Raghupathy, J. Neurochem. 19, 1423 (1972).

3. J.P. Bennett, Jr., W.T. Logan and S.H. Snyder, J. Neurochem. 21, 1533 (1973).

4. N.A. Peterson and E. Raghupathy, J. Neurochem. 21, 97 (1973).

5. W.J. Logan and W.H. Snyder, Brain Res. 42, 413 (1972).

6. S.H. Snyder, A.B. Young, S.P. Bennett and A.H. Mulder, Fedn. Proc., 32, 2039 (1973).

7. R.B. Loftfield and E.A. Eigner, Science, N.Y. 164, 305 (1969).