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Inhibition by L-Phenylalanine of Tryptophan Transport by Synaptosomal Plasma Membrane Vesicles: Implications in the Pathogenesis of Phenylketonuria

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Phenylalanine is accumulated in the genetically linked deficiency phenylketonuria. The effect of L-phenylalanine on the transport of tryptophan was studied using membrane vesicles from rat-brain synaptosomes. Phenylalanine at similar concentrations to those found in phenylketonuric patients competitively inhibits tryptophan uptake, with a K_i of the same order as the K_m for tryptophan. This inhibition could be responsible for the depletion of serotonin found in phenylketonuria.

Although the basic biochemical defect in phenylketonuria—the genetically linked deficiency of the hepatic phenylalanine hydroxylase system—is well characterized, the mechanisms by which increased phenylalanine causes brain dysfunction are still a subject of research. Several lines of experimental evidence suggest two principal causes for the brain dysfunction in phenylketonuria: defective myelination and impairment in the synthesis of neurotransmitter amines (for a review see Blau, 1979).

In patients with phenylketonuria an abnormal indole metabolism in general, and impairment in the serotonin metabolism in particular, as well as significantly depleted serotonin concentrations, have been found (Vorhees *et al*., 1981; McKean, 1972). The serotonin depletion, which appears to be related to the high increase of phenylalanine levels, has been proposed as a current cause for the pathogenesis of brain dysfunction in phenylketonuria.

On the other hand, because the concentration of the aromatic amino acid tryptophan, precursor of serotonin in brain cells (McKean *et al*., 1968; Tyfield and Holton, 1976) is relatively small when compared to the concentration of most other amino acids, it appears that the transport process across plasma membranes might be the rate-limiting step controlling its concentration (Grahame-Smith and Parfitt, 1970; Lajtha, 1974).

In our laboratory we have demonstrated that membrane vesicles derived from brain synaptosomes transport tryptophan by an Na^+-gradient-dependent system analogous to those previously described for other amino acids in similar preparations (Kanner, 1978; Kanner and Sharon, 1980; Aragon *et al*., 1981, 1982; Mayor *et al*., 1981; Marvizon *et al*., 1981).

The presence of high- and low-affinity transport systems for neurotransmitter amino acids and neurotransmitter precursors has been demonstrated in different preparations of brain (Logan and Snyder, 1972; Kuhar and Zarbin, 1978; Mandell and Knapp, 1977; Aragon *et al*., 1981). High-affinity Na^+-dependent systems for tryptophan uptake are thought to be implicated in the maintenance of appropriate tryptophan levels in the nerve cell to ensure serotonin synthesis (Fernston and Wurtman, 1971; Grahame-Smith, 1971), whereas the low affinity system would subserve general metabolic functions (Hedqvist and Stjarne, 1969; Johnston and Iversen, 1971).

The results indicate that phenylalanine at physiopathological concentrations does inhibit the uptake of tryptophan by membrane vesicles derived from rat brain synaptosomes. This appears to be the most likely cause for the serotonin depletion in phenylketonuria.

MATERIALS AND METHODS

L-[G-^{3}H] Tryptophan (sp. radioactivity 3.1 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., UK. Ficoll 400 was obtained from Pharmacia. All other materials were of the highest purity available.

Adult male rats of the Wistar strain, weighing 150–200 g, were used. Membrane vesicles were isolated from rat brain essentially as described previously (Kanner, 1978; Aragon *et al*., 1981).

L-Tryptophan uptake was determined by a filtration technique. Portions (20 μl) of the suspension of membrane vesicles (about 0.1 mg of protein), preloaded with 120 mmol/l KCl–22 mmol/l potassium phosphate buffer, pH 7.4 (KCl medium), were pre-incubated for 1 min at 25°C. Uptake was started by adding 100 μl of a solution containing L-[G-^{3}H] tryptophan (5 μmol/l final concentration) in 120 mmol/l NaCl 22 mmol/l sodium phosphate, pH 7.4 (NaCl medium) or in KCl medium. The experiment was terminated by diluting with 5 ml of ice-cold 0.8 mol/l NaCl, and immediately filtering through a moistened Millipore filter, RAWP 02500 (1.2 μm pore size), attached to a vacuum assembly. The filters were rinsed twice with the ice-cold medium

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Dilution, filtration and washing procedures were performed within 15 s. The filters were dried at 60°C, placed in microvials and their radioactivity was measured in a liquid-scintillation counter (Beckman LS-350). All the experiments were corrected for a control obtained by diluting the membrane suspension before adding the radioactive substrate solution. All solutions used in the preparation of the membrane vesicles and in the uptake experiments were prepared with distilled and de-ionized water and had been filtered through Millipore filters (0.45 µm) to avoid possible bacterial contamination. The osmolarity of all solutions was kept constant during the experiments. The pH of external and internal medium was 7.4 throughout the experiments.

Membrane proteins were determined according to the method of Resch *et al.* (1971).

RESULTS

Aromatic amino acids interact in their influx into brain cells both *in vivo* (Guroff and Udenfriend, 1962; Blasberg and Lajtha, 1965; McKean *et al.*, 1968) and *in vitro* (Neame, 1961; Pratt, 1976; Barbosa *et al.*, 1970; Vahvelainen and Oja, 1975). Although common mechanisms for transporting aromatic amino acids have been proposed on the basis of this mutual interference, it is questionable whether they share a common transport system (Vahvelainen and Oja, 1975; Lähdesmäki and Hannus, 1977).

The effect of L-phenylalanine on the uptake of L-tryptophan by synaptosomal plasma membrane vesicles from rat brain, has been tested at concentrations similar to those found in the plasma of phenylketonuric patients (Figure 1). As shown in Figure 1A phenylalanine inhibits the tryptophan uptake, both in the presence of a Na^+ gradient, as well as under non-gradient conditions (i.e. KCl medium). Figure 1B shows the effect of phenylalanine on the specific Na^+-dependent tryptophan uptake. Over the past few years, the existence of two processes of uptake, sodium dependent, in brain preparations, with high and low affinities for tryptophan have been postulated (Grahame-Smith and Parfitt, 1970; Mandell and Knapp, 1977; Laakso and Oja, 1979; Korpi, 1980). The kinetic data of the high-affinity system (K_m = 35 µmol/l) in the presence of a Na^+ gradient and 10 and 40 µmol/l L-phenylalanine in the medium are shown in Figure 2. The analysis of these data by Lineweaver-Burk plots demonstrates that L-phenylalanine is a competitive inhibitor for the tryptophan uptake with a K_i of 41 µmol/l. Phenylalanine at concentrations of 2.5 mmol/l, also inhibits the low-affinity system (K_m = 1.46 mmol/l) competitively with a K_i of 1.50 mmol/l (data not shown).

DISCUSSION

Because the membrane vesicles preparation allows the use of a well-defined ionic environment and energy sources and avoids metabolic and compartmentation interference, the nature and kinetic properties of the transport systems for aromatic amino acids in the brain can be studied more effectively. Results support the

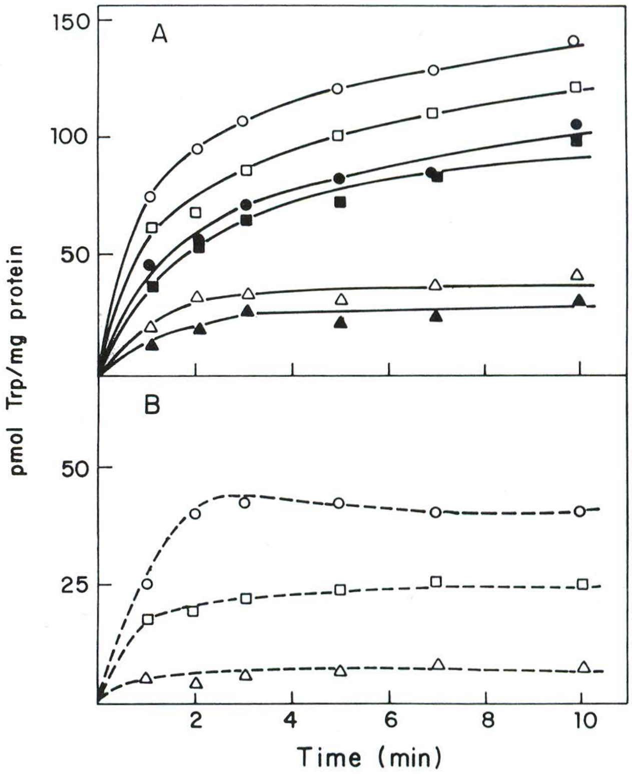

Figure 1 Effect of phenylalanine on tryptophan uptake by membrane vesicles from rat brain. The vesicles were preloaded with KCl medium, and incubated as described in the Materials and Methods section in the presence of 5 µmol/l L-[G-^{3}H] tryptophan and 0 (○, ●), 10 (□, ■) or 500 (△,▲) µmol L-phenylalanine in NaCl (○, □, △) or KCl medium, (●,■, ▲). The compositions of the media are described in the Methods section. The specific Na^+-dependent tryptophan uptake (dashed lines) was obtained by subtracting the uptake in the NaCl medium (○, □, △) from that in the KCl medium (●, ■, ▲) at each time

postulate that phenylalanine and tryptophan have common transport systems (Blasberg and Lajtha, 1965; Sershen and Lajtha, 1979) since they inhibit to a considerable extent each others' influx competitively. The inhibition constants calculated from various different inhibitor concentrations (Figure 2), are of the same order of magnitude as the K_m value. These results are consistent with the idea that the influx of several amino acids will be reduced in children with phenylketonuria (Pratt, 1981). Interference in the transport of tryptophan by a large excess of phenylalanine, as demonstrated in the present report using a membrane vesicles preparation, might be of significance in phenylketonuria where phenylalanine accumulates in the tissues and body fluids of patients. These increased concentrations of phenylalanine, by decreasing the availability of the precursors tryptophan and tyrosine (Aragón *et al.*, 1982), might be the primary cause of serotonin and catecholamine depletion in phenylketonuria (Figure 3).

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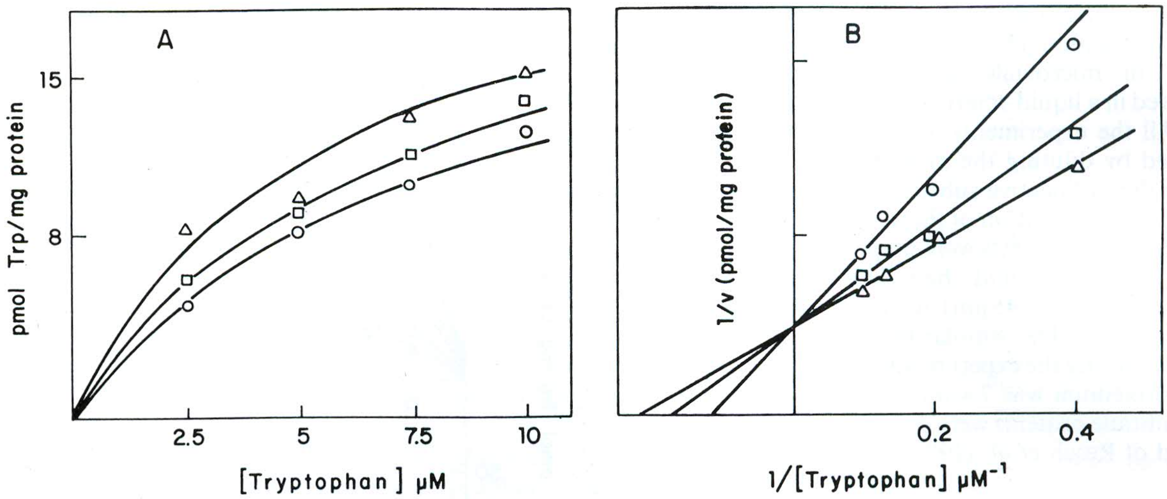

Figure 2 Kinetic and double reciprocal plot of initial (30 s) uptake of tryptophan with various concentrations of tryptophan in the presence of 0 (△), 10 (□) or 40 (○) μmol/l phenylalanine. The membrane vesicles, preloaded with KCl medium, were incubated for 30 s as described in the Materials and Methods section in the NaCl medium and radioactive tryptophan at the concentrations indicated. Lineweaver-Burk plots have been generated using the least-squares fitting method.

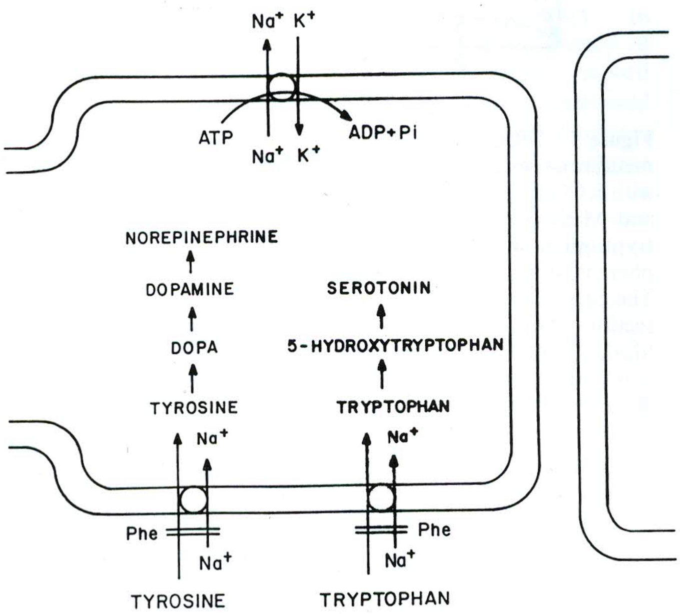

Figure 3 Phenylalanine interference on the transport of tryptophan and tyrosine in neuron-presynaptic membrane.

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