Inhibition by Phenylalanine of the Entry of 5-Hydroxy-Tryptophan-1-C^{14} into Cerebrospinal Fluid.* (29792)

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There is considerable evidence indicating that naturally-occurring amino acids are actively taken up by brain slices, that this uptake is stereo-selective, and is inhibited by elevated concentrations of other amino acids in the suspending medium(1-5). Similar effects have been demonstrated under *in vivo* conditions for tyrosine and 5-hydroxytryptophan (5-HTP)(6,7) although, in general, the brain *in vivo* takes up amino acids more slowly than other tissues(4). Since amino acids are water soluble polar molecules that would not ordinarily be expected to pass the blood-brain barrier, the concept has evolved that there are specific transport mechanisms available for uptake of amino acids by the brain(8). Disturbances in circulating levels of one or more amino acids are thought to exert effects on transport of other amino acids into the brain.

In addition to the study of naturally-occurring amino acids, the use of C^{14} labelled compounds had indicated that there is a rapid exchange of free amino acids between plasma and brain(9). In particular, 5-HTP-C^{14} has been used for studying the effects of a variety of substances, including other amino acids, on the influx of this amino acid into brain slices(10,11) or into brain *in vivo* (7). L-phenylalanine was shown to be one of the most potent amino acids exerting an inhibitory effect on the entry of 5-HTP-C^{14} into brain.

One of the difficulties involved in interpretation of results from the study of brain homogenates is the variety of compartments present in the central nervous system (CNS). In addition to the regional variations in different CNS areas, there are the interstitial fluid spaces, the variety of cell types and the subcellular compartments in individual cells. Data from homogenates of whole brain can only be considered as the mean of these separate components.

The view is now generally held that cerebrospinal fluid (CSF) may be looked upon as representative of CNS interstitial fluid (12). This study reports the effects of elevated plasma phenylalanine levels on the entry of 5-HTP-C^{14} into CSF of anesthetized dogs. The use of CSF in this manner has the advantages of limiting analysis to a single, homogeneous CNS compartment and also of allowing study of blood-brain barrier transport systems without sacrificing the subject.

Methods. Mongrel dogs (15-20 kg) of either sex were used. The animals were anesthetized with 30 mg/kg of intravenously administered pentobarbital sodium with added supplements as needed. Arterial blood was obtained from a cannulated femoral artery. A spinal trocar was inserted into the cisterna magna and left *in situ* throughout the experiment. Three microcuries/kg of DL-5-hydroxytryptophan-11-C^{14}† were administered into a cannulated femoral vein. Four dogs served as controls. Six dogs received 0.35 or 0.7 g/kg of L-phenylalanine in a 0.15 molar solution into the opposite femoral vein over a one-hour period prior to administration of the isotope. Two dogs were pretreated with 0.7 g/kg of D-phenylalanine in a similar manner.

Blood and CSF samples were obtained simultaneously at stated intervals. The blood was collected in heparin and plasma separated immediately after centrifugation. Negligible radioactivity was present in the erythrocytes.

A spectrophotofluorometric method(13)

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† Obtained from Calbiochem., specific activity 2.85 mC/mM.

was employed for phenylalanine in plasma. This method was modified slightly for CSF.

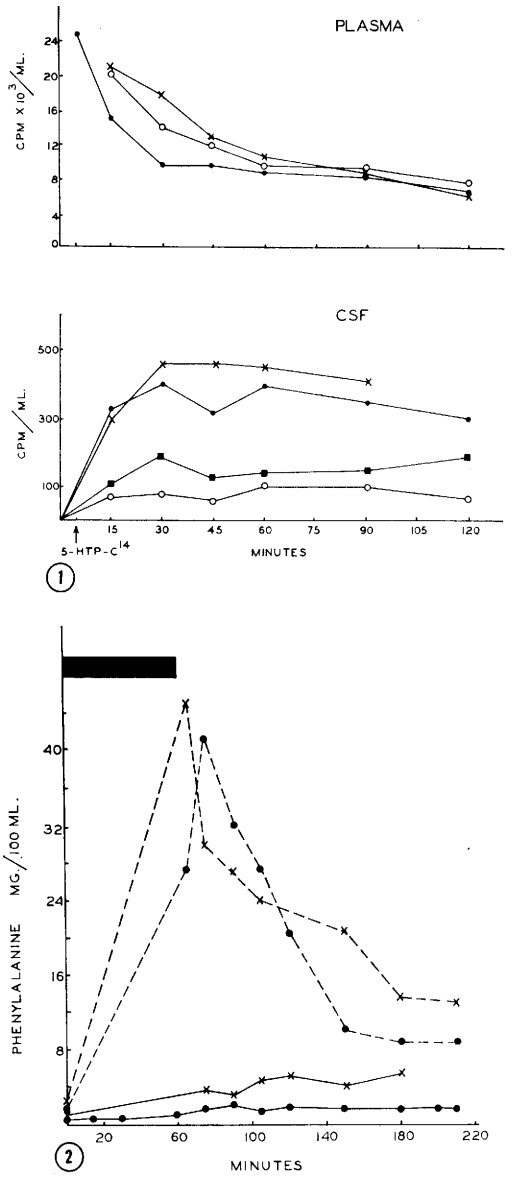

FIG. 1. Effect of pretreatment with phenylalanine on entry of intravenously administered 5-HTP-C^{14} into CSF. ●——●, Control (4); ■——■, L-phenylalanine 0.35 g/kg (3); ○——○, L-phenylalanine 0.7 g/kg (3); ×——×, D-phenylalanine 0.7 g/kg (2); CPM, mean counts per min. (Numbers in parentheses indicate No. of experiments from which mean CPM were derived.)

FIG. 2. Levels of phenylalanine in plasma and CSF after phenylalanine infusion. The bar refers to intravenous administration of 0.7 g/kg of phenylalanine. -----, Plasma; ——, CSF; ×, L-phenylalanine; ●, D-phenylalanine.

All samples containing radioactivity were determined by conventional methods(14) using a liquid scintillation spectrometer.

Results. The entry of 5-HTP-C^{14} into CSF and its decay in plasma in these experiments are illustrated in Fig. 1. There is a significant inhibition of entry of the isotope into CSF in the animals pretreated with L-phenylalanine. Pretreatment with D-phenylalanine did not exert a similar effect. The decay of isotope in plasma was apparently not affected by pretreatment with phenylalanine.

Fig. 2 illustrates two experiments in which the levels of phenylalanine were determined in plasma and CSF. There is a rise in CSF phenylalanine after intravenous administration of 0.7 g/kg of L-phenylalanine indicating passage of this amino acid into the CNS.

Discussion. These experiments show that a high plasma level of L-phenylalanine inhibits the entry of isotopically-labelled 5-HTP into CSF. This is in accord with previous findings in rats that administration of phenylalanine interferes with the transport of this isotope into brain(9). These findings support the view that disturbances of transport of amino acids into the CNS can be detected by examination of the CSF although further data are required regarding correlations of CSF and brain levels. Whether there is a relationship between these effects and the behavioral and neurological abnormalities occurring in experimental and clinical phenylketonuria is not clear.

Some caution must be exercised in inferring effects of substances on net transport mechanisms by the use of radioisotopes. It has been reported that there are efflux mechanisms regulating amino acid concentrations in brain causing amino acids to pass from brain to blood against a gradient of elevated plasma amino acid levels(15). Recent studies indicate that elevated blood levels of certain amino acids may actually increase the concentration of other amino acids in CSF(16, 17). This raises the possibility that high plasma amino acid levels may affect efflux as well as influx rates of other amino acids in the CNS. If inhibition of the efflux rates should predominate, an increase of CNS

levels of other amino acids would be expected to occur.

Summary. High plasma levels of L-phenylalanine inhibit the entry of 5-hydroxytryptophan-C^{14} into the CSF of dogs. CSF is an accessible body fluid that may be used to study transport of amino acids into the CNS.

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Characterization of Prototype Virus ECHO-32. (29793)

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During studies of infectious agents isolated from Puerto Rican infants with diarrhea(1) several viral agents were recovered which appeared to conform to criteria established for ECHO viruses, but did not belong to any known ECHO group. Two of these agents have been fully characterized and submitted to the Committee on Enteroviruses. One of the viruses, PR17, was reported by the Committee to be related to ECHO virus type 30, the prototype of which is Bastianni(2). The other, PR10, was accepted as the prototype strain of ECHO-32(3). The following report presents characteristics of this virus.

Materials and methods. Source. This agent was isolated from a 20-month-old female admitted with diarrhea, nausea and fever of 3 days' duration. Examination revealed a well-nourished, alert, and well-hydrated infant with moderate upper respiratory distress and a temperature of 101°F. Pertinent laboratory findings included a white count of 21,155 cells/cu mm and numerous *Giardia lamblia* cysts, *Escherichia coli* 019 and Enterococci in her stool. Convalescent phase blood was drawn two weeks later.

Tissue culture. Methods for recovering viruses from fecal sample during this study have been previously described(1). Briefly, the fecal sample was mixed with physiological saline to make approximately a 20% suspension and centrifuged at 3000 rpm for 1 hour at 4°C. One hundred μg of streptomycin and 1000 units of penicillin G were added and this preparation utilized to inoculate rhesus monkey kidney tissue cultures (MKTC). The cultures were maintained in bovine amniotic fluid containing 0.005% phenol red, 100 μg streptomycin and 100 units of penicillin/ml.

Identification. Virus pools were prepared and neutralization tests carried out in cell cultures just described. Antisera for neutralization tests against ECHO viruses types 1