Brain Uptake of $^{11}$C-Methionine in Phenylketonuria

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Abstract. The brain uptake of $^{11}$C-methionine was studied in 26 children with classical phenylketonuria; one adult was used as a control. Labelled methionine uptake in brain was first measured during a low phenylalanine diet and again one week later after a load of phenylalanine. Ten children aged 1 to 30 months were studied twice at intervals of several months. In children having a phenylalaninemia ≥0.3 μmoles · ml⁻¹, a decrease in methionine brain uptake was observed with increasing age, with the largest change occurring during the first year of life. After the phenylalanine load, a mean increase in phenylalaninemia by a factor of ten was accompanied by a mean decrease in brain methionine uptake by a factor of two while blood methionine remained unchanged. Brain activity curves increased with time for children younger than one year and having phenylalaninemia < 0.6 μmoles · ml⁻¹. After the age of 2 most patients had a decreasing curve regardless of the blood phenylalanine level. This study indicates that $^{11}$C-methionine brain uptake may be taken as an index of blood brain barrier permeability to essential amino acids, and of brain maturation. The results obtained suggest that an increase in phenylalaninemia to levels greater than 0.6 μmole · ml⁻¹ induces a modification in brain uptake of amino acids, primarily during the first two years of life.

Key words: Blood-brain-barrier - $^{11}$C-methionine - Phenylketonuria

Introduction

Phenylketonuria (PKU) is characterized by a phenylalanine hydroxylase deficiency and a consequent increase in blood phenylalanine concentration (B Phe). Children suffering from this congenital disease develop irreversible mental retardation which can be prevented by placing them on a low Phe diet. However, it is not accurately known at what level B Phe should be maintained, nor is it known at what age the diet should be ended in order to be sure that mental retardation does not develop. A collaborative study comparing results in two groups of children equilibrated between 0.06 to 0.3 μmole · ml⁻¹ and 0.33 to 0.6 μmole · ml⁻¹ has been undertaken to answer this question [21].

Essential amino acids enter brain tissue by a saturable transport system [22] and it has been shown that an excess of blood phenylalanine, which disturbs the passage of other essential amino acids through the blood-brain barrier (BBB), leads to a modification of the free amino acid pool and a decrease in brain protein synthesis, especially in myelin [1].

A method developed to measure the brain uptake index in rats allowed Oldendorf [18] to quantify the decrease in the penetration of essential amino acids through the blood-brain barrier (BBB) when an excess amount of phenylalanine was administered. Of these, phenylalanine caused an 80% decrease in penetration of methionine through the blood-brain barrier. Oldendorf et al. also showed [19] in phenylketonuric adolescents and adults a decrease in brain penetration of selenomethionine-$^{75}$Se compared with non-phenylketonuric mentally retarded patients. Quantification of this phenomenon however has been difficult because the long half-life of $^{75}$Se necessitates low doses to minimize radiation to the patient, and the low gamma energy causes uncertainty due to attenuation by tissues.

In order to measure more accurately the relation between phenylalaninemia and brain uptake of amino acids, the brain distribution kinetics of methionine-$^{11}$C were studied in young children with PKU.
Materials and Methods

Carbon 11 was used as a tracer because of its short half-life (20 min) and because it can be accurately measured using an amount of activity which gives a radiation dose of 0.1 Rad/mCi to a child weighing 10 kg. This dose is comparable to a radiographic examination of the thorax. In addition, because it disintegrates by emitting positrons, carbon 11 emits a 511 keV gamma ray which can be detected by external counting. A technique for synthesizing methionine-\textsuperscript{11}C was developed [6] consisting of methylating L-homocysteine with \textsuperscript{11}CH₃.

The specific radioactivity at the moment of injection was 400 to 1000 mCi/μmole. The chemical and radiochemical purity of the preparation was greater than 85% for the first ten examinations and greater than 99% for the subsequent ones. No radiolysis was observed chromatographically for at least one hour after the methionine had been prepared. The choice of the labelling position was governed by the half-life of the radioisotope, and also allows production of pure L-methionine rather than a racemic mixture.

The study dealt with 26 phenylketonuric children between the ages of 1 month and 6 years, and one normal adult; ten were examined twice within a minimum interval of 4 months. All the children in this study were classic phenylketonurics (blood phenylalanine $\geq 1.5$ μmoles · ml$^{-1}$ with protein intake of 3 g/kg/24 h). All were placed on a low phenylalanine diet before the age of 3 months and all were within the normal range of mental development. These studies were undertaken with the informed consent of the parents.

The test consisted of two parts: the first was an examination of children on a diet low in phenylalanine; the second one week later in which two oral loads of 150 mg/kg of phenylalanine were administered 18 h and 1 h before the examination. During the two days following this second examination of phenylalanine-free diet was given to the children so that blood phenylalanine (B Phe) returned to normal within two to four days. For two children aged 1 and 1.5 month the procedure was reversed.

Half hour before the beginning of the examination the children were given 1.5 g of chloral/m$^{-2}$ per body surface by mouth and were placed before a gamma camera equipped with a high energy collimator. The head of each patient was viewed in profile by the detector crystal. 100 to 125 μCi/kg body weight of \textsuperscript{11}C-methionine were injected in a peripheral vein and the head radioactivity was recorded in list mode during 20 min. Because of the short half-life of carbon 11 and the low amount of radioactivity injected it was not possible to follow the brain radioactivity curve longer than 20 min. The amount of cold methionine injected with the labelled molecule (less than 2.5 nmole) was negligible compared to the normal blood methionine concentration, and thus could not have significantly affected the brain uptake of the radioactive tracer.

Blood phenylalanine and methionine were measured in samples taken at the time of injection of the radioactive tracer [5]. Blood methionine concentration was found to be normal in all cases (0.83 μmole/100 ml±5% S.D.) and did not change significantly after the phenylalanine load.

To allow expression of the results as a percentage of the injected dose, a brain phantom containing a known amount of carbon 11 was measured under the same conditions as the heads of the children and compared to the head radioactivity corrected for radioactive decay.

Effective knowledge of brain uptake requires correction for the radioactivity of the blood, scalp and bone tissue surrounding the brain which are measured along with it. The principle of such correction, described elsewhere [7], is based on the hypothesis that the only correction parameter varying from one examination to another in the same child, (when he is on a diet low in phenylalanine and when he is not), is that of brain tissue radioactivity.

This assumption was presumed to be valid since the blood radioactivity curves following i.v. injection of \textsuperscript{11}C-methionine were identical for both tests.

On pictures of the head obtained during the first and second tests, 2 regions of interest—one of the whole brain (A) the other of a small region at the center of the brain (B)—were chosen. From the radioactivity at any time in these two regions and for each two tests, the extracerebral radioactivity ($x$) was calculated using the relation:

$$x = \frac{m_2 n_1 - m_1 n_2}{n_1 - n_2 - R (m_1 - m_2)}$$

where $m_1$ and $m_2$ are the activities of region A for the two tests, $n_1$ and $n_2$ the activities of B for the two tests and R the ratio of the volumes of the extracerebral tissue in A and B. These volumes were measured on X-ray pictures of the head of the patients assuming the head is of ellipsoidal shape.

Results

The appearance and evolution of cerebral radioactivity after i.v. injection of \textsuperscript{11}C-methionine are due to various phenomena: transport through the blood-brain-barrier, extracellular distribution, blood exchange, and cellular incorporation. The global evolution of these parameters expressed by the methionine brain uptake was followed according to the age and degree of phenylalaninemia of the children studied.

As an example, the difference in the amount of methionine-\textsuperscript{11}C penetrating the brain and in its distribution kinetics when phenylalaninemia is increased are shown on the gamma camera photos and the curves in Fig. 1 experimentally obtained in a 12 month old child.

When B Phe is low (first test while on the diet) a rapid increase of radioactivity during the first minutes (uptake) is observed and the curve of brain activity shows a gradually increasing slope. When B Phe is high, a much lower brain uptake of methionine-\textsuperscript{11}C is observed in the first minutes after injecting the tracer, followed by a decreasing slope. This decrease in brain radioactivity with time corresponds to a disappearance of the tracer from the field of the camera.

Table 1 shows the results for the 26 children and 1 adult studied, classified by increasing age. The data given in this table cover both brain uptake and kinetics of \textsuperscript{11}C-methionine.

Methionine-\textsuperscript{11}C Uptake

The uptake of methionine-\textsuperscript{11}C varied with two parameters: the degree of phenylalaninemia and the age of the child.