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Amino Acids Labelled with ^{11}C as Indicator of the Effect of Dietary Treatment of Hyperammonaemia

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ABSTRACT

Short-lived radioactive carbon, ^{11}C, ($T_{1/2}$=20 min) was incorporated into an essential amino acid [^{11}C-methyl]-L-methionine, to form a true biological amino acid tracer with external detectability. This was tested in a study of the physiological tracer dynamics in a hyperammonaemic patient before and after a change in the dietary treatment. The protein intake was unchanged between the two investigations but the energy intake was increased from 53 to 63 kcal/kg BW/day. The tracer radioactivity was given per os. In the second investigation a relative decrease of radioactivity in the low molecular weight fraction of blood plasma was seen. Also the external measurements indicated a higher hepatic retention of radioactivity in the second investigation but no increased excretion of tracer. This may reflect an increased ability of the liver to utilize the incoming methionine from the vena porta. The hyperammonaemia remained over the second investigation but seven months later the ammonia content in the blood was almost normalized and the patient had also gained 3 kg in weight. The correlation between changes in tracer dynamics and changes in therapeutical effect of the diet is not further verified in this experiment but the investigation indicates the value of further studies in this topic using ^{11}C-labelled amino acids also including the use of the newly introduced positron tomographic technique. It may be possible to develop this type of nuclide technique further to achieve a clinically useful method of optimizing therapeutic regiments in this type of metabolic disease.

INTRODUCTION

^{11}C is a short-lived positron-emitting radionuclide yielding 511 keV annihilation photons which allows external detection. The short physical half-life of ^{11}C, 20 minutes, implies that the radioactivity can only be traced for a few hours after administration to the subject. In a study performed on pigs ^{11}C-radioactivity was followed in blood, liver, muscle and exhaled air after intravenous administration of [^{11}C methyl]-L-methionine (11). When the methionine content of the pig feed was changed, responses in the measured variables could be shown. The conclusion from this animal study was that it seems likely that ^{11}C-labelled methionine is a useful tracer of amino acid metabolism. In this paper the [^{11}C-methyl]-L-methionine used is sometimes referred to as ^{11}C-methionine.

^{11}C-methionine has also been used in the investigation of lesions of the human brain, in patients with phenyl-ketonurea, with positron emission tomography (2). This study showed a changed uptake of ^{11}C in the brain when the protein balance was changed. Unfortunately, the data presented did not reveal the actual changes in the protein balance.

Several human inborn errors of metabolism are treated dietarily. Hyperammonaemia (i.e. urea cycle disorders resulting in toxic ammonia levels in blood) are treated with a protein-balanced diet (9,4) which decreases the hyperammonaemia and the toxic symptoms of the metabolic disease. It is difficult but nevertheless important to keep the patient in an anabolic condition to minimize the risks of hyperammonaemia. Repeated investigations of a hazardous nature are neccesary to evaluate the protein and energy balance. The aim of this study was not quantitatively to describe the pathology but primarily to test the use of ^{11}C-labelled amino acids for the investigation of patients with nutritional disturbances.

SUBJECT

A girl born 1977. From the first years of life she chose a protein restricted diet and weaning was extremely difficult. At the age of 2 years she suddenly became unconscious and remained so during one day. When she woke up she had a cerebellar ataxia for several days and improved slowly. Extensive investigations were performed and during a 'protein provocation' (1.5 g protein/kg BW) for 36 hours she developed signs of intoxication with ataxia and hyperammonaemia. The amino acids in plasma and urine did not point to the exact diagnosis. As patients with this kind of disease are vulnerable from the metabolic point

of view we have deliberately avoided further provocations and biopsies. No new attacks of severe hyperammonaemia have occured during the treatment with protein and energy-balanced diet. However,there have been periods of too low energy intake resulting in slight hyperammonaemia. During such a suspected period these investigations were performed.

The first investigation was performed in April 1980 during an intake of 53 kcal/kg BW/day and a protein intake of 0.7 g/kg BW/day. The second investigation was performed one month later during an intake of 63 kcal/kg BW/day and unchanged protein intake.

MATERIAL AND METHODS

Production of[^{11}C-methyl]methionine

The radionuclide ^{11}C was produced at the Tandem Accelerator Laboratory in Uppsala (TLU). The radionuclide was incoporated into the methyl group of methionine according to (7,8). The preparation was used after pH-adjustment and sterile filtration through Millipore filter (0.22 µm). Analysis on a LC reverse phase system (C-18 column with water/methanol as eluent) of the solution obtained showed that the labelled methionine was obtained in radiochemical purity better than 99.5%. In this investigation there was no separation of the inactive homocysteine (at most the amount of 15 mg, i.e. 0.1 mmol in the whole prepared volume). The specific radioactivity of the labelled methionine was about 370 MBq/mmol(10 mCi/mmol) as estimated from determinations of the specific radioactivity of the[^{11}C-methyl]iodide used.

Detectors and data handling

Samples of blood, exhaled CO_2 and urine were measured in a NaI(Tl)-crystal detector of well type. For external detection cylindrical NaI(Tl)-crystal detectors (2" * 3") were used to detect ^{11}C externally in chosen structures. All detectors were connected to single channel analysers set to detect 511 keV events (annihilation photon energy) only. Counts accumulated for one minute were transferred to a data log (Texas Silent 700 Terminal with cassette tape deck) for off-line computer evaluation.

For normalization $Na_2{}^{11}CO_3$ dissolved in a water phantom viewed by the external detectors was used. A sample from the phantom fluid was measured in the well type detector and the relative sensitivity of all detectors was thus obtained. In investigations of the patient an aliquote of the radioactivity

administered to the patient was also measured in the well type detector. The radioactivity measured by the external detectors could then be normalized to permit calculation of the fraction of the radioactivity administered in the observed tissue volume and its deviation from conditions of homogenous dilution in the body. The observed radioactivity was corrected for background, radioactive decay, detector effeciency and dead time losses. The standard error of the normalized values was also calculated.

Measurements of radioactivity in the investigated subject

At the start of the measurements 12 MBq (0.33 mCi) of [^{11}C-methyl] methionine was given per os in a glass of fruit drink to the fully conscious patient weighing 24 kg. This amount of tracer allowed measurements during a 2 hour period. The high specific radioactivity of the tracer implies no change in the physiological plasma concentration of methionine.

Blood samples were taken from a cubital vein for the analysis of amino acids and ammonia as well as for the measurement of radioactivity. After measuring radioactivity in whole blood the samples were centrifuged to separate the blood cells from plasma. Blood plasma was separated into high and low molecular weight fractions by gel chromatography on Sephadex G25 columns (PD-10 pre-prepared columns, Pharmacia, Uppsala, Sweden).

Exhaled air was at intervals sampled by a scavenger pump connected to a modified mask of narcosis (only used for air sampling). The sampled air was passed trough a CO_2 absorbant (Ascarite, Thomas Company, Philadelphia, PA, USA) which after a sampling period of 1-2 minutes was measured in the well type detector giving the amount of exhaled radioactivity per minute.

The patient was asked to empty the bladder before the start of the measurements. After two hours the urine produced was collected and measured for radioactivity.

Radioactivity in liver and muscle areas was externally detected. The liver and the stomach detectors were slit collimated by heavy lead shielding (5 cm thick with a 3 * 0.5 cm slit opening) and placed immediately close anterior to the patient who was in a half-sitting position. The third detector was placed 10 cm under the upper part of both legs watching these through a slit collimator opening 1 * 10 cm at 10 cm distance.

The design of the data collecting is illustrated in Fig. 1.

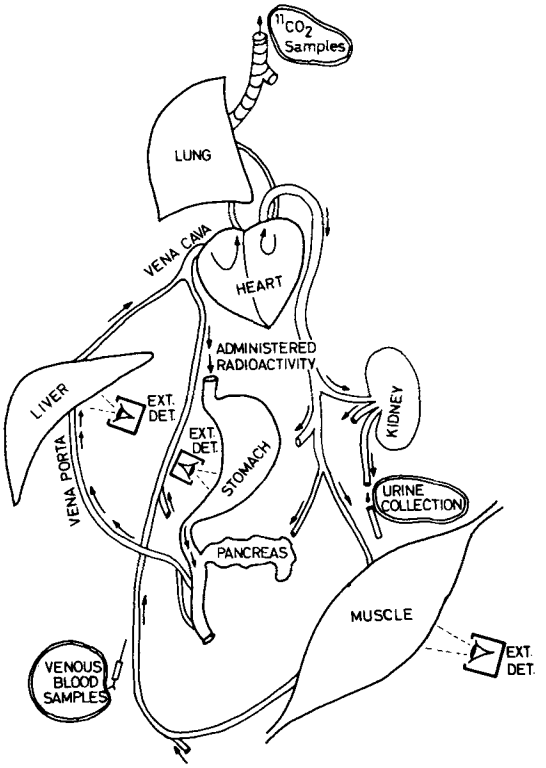

Fig. 1. The figure illustrates schematically the main organs concerned, radioactivity transportation path (blood) and the different sites of measurements (SAMPLES + EXT. DET.). The radioactivity was administered per os. When entering the stomach the radioactivity was externally detected. It was taken up from the intestine and transported in the vena porta to the liver where it was detected externally. Radioactivity that escaped from the liver was distributed to other organs and was thus also detected over muscle tissue. Samples of venous blood were taken and analysed as well as samples of exhaled carbon dioxide and urine.

Radiation dose to the investigated subject

The absorbed dose of ionizing radiation from ^{11}C was calculated from physical data on ^{11}C decay and dynamic data on ^{75}Se-methionine published in the "MIRD-series" of the Journal of Nuclear Medicine (3,5,10). These calculations which were presented to the Isotope Committee, Uppsala University Hospital, showed an expected whole body dose of 2 μGy/MBq (8 mrad/mCi) of

^{11}C-methionine given to an adult man of 70 kg BW. Critical organs proved to be liver, pancreas, intestine, some glands and stomach.

RESULTS

Blood ammonia and weight increase

At the beginning of the study the ammonia level in blood was about 165 µmol/l (normal value is below 70 µmol/l). At the second investigation the increase in energy intake implied only minor changes in blood ammonia. However, in the long term follow up 7 months later, with the patient still on the same raised energy intake, the blood ammonia had almost normalized (104 µmol/l). There was a gain in weight of about 3 kg during these seven months.

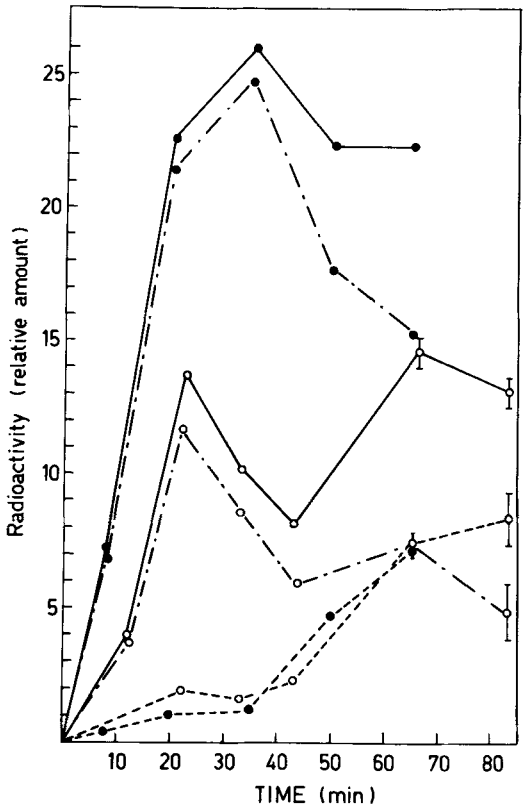

Fig. 2. The figure shows the total plasma radioactivity as a continuous line (—), the low molecular weight fraction ´dash-dot-dash´ (-.-.-) and the high molecular weight fraction with broken lines (- -). The first investigation (low energy intake level) with filled circles (●) and the second investigation (incresed energy intake) with unfilled circles (o).

Tracer distributions

Blood: The radioactivity in whole blood was higher in the first study. The erythrocyte volume fraction was the same in both studies. The difference in radioactivity was mainly due to a difference in the low molecular fraction (Fig. 2).

Exhaled carbon dioxide radioactivity: No clear difference was shown in the two investigations, although a slightly higher radioactivity seemed to be present in the first investigation.

Urine: No marked difference in radioactivity in urine was shown.

Stomach: The shape of the curves showing the emptying of the stomach are almost equal in both studies (Fig. 3).

Liver: In the second investigation the total radioactivity in the liver was found to be higher indicating an increased retention of radioactive substance (Fig. 3).

Muscle:The radioactivity was found to be higher in the first study (Fig.3).

The radioactivity measured in blood plasma fractions are shown in Fig. 2. The radioactivity in the erytrocyte fraction followed largely the values of total plasma. The radioactivity externally detected over stomach, liver and muscle is shown in Fig. 3.

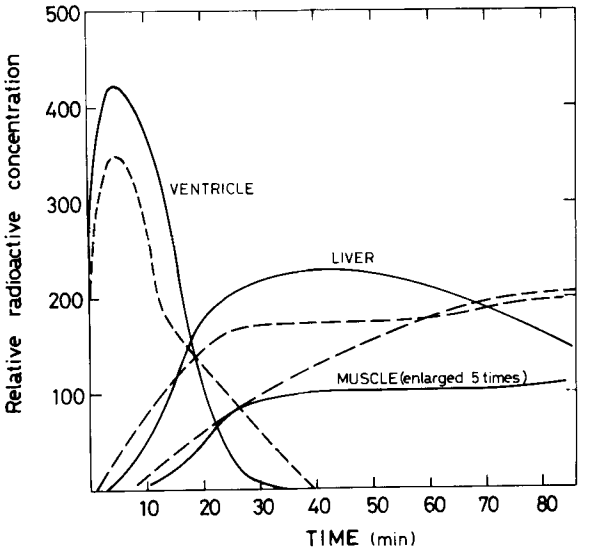

Fig. 3. The figure shows the externally detected radioactivity over stomach, liver and muscle. The radioactive concentration measured was normalized to 100 at conditions of homogenous dilution. The interrupted line (- -) shows the measurements of the first investigation (low energy intake level). The continuous line (—) shows the measurements of the second investigation (increased energy intake).

The changes between the two studies are summarized in Table 1.

Table 1. Changes in the detected radioactivity between the two investigations.

Measured parameter	Change
Liver	+
Muscle	-
Blood plasma	
low mol. w. fraction	-
high mol. w. fraction	0
Exhaled carbon dioxide	0
Urine	0

DISCUSSION

The tracer is after per os administration taken up via the intestine into the blood and transported in the vena porta to the liver. In the liver the tracer is to a large extent retained. The liver is the organ responsible for the degradation and the oxidation of methionine. Plasma proteins are to a great extent synthesized in the liver. With whole-body autoradiography the radioactivity accumulation in different organs has been studied in mice after i.v. injection of ^{11}C-methionine (1). Radioactivity uptake can then be seen in: liver, pancreas, intestinal wall, salivary glands and bone marrow. The external end-compartments urine and exhaled carbon dioxide give information on the momentary excretion (11). Analysis of blood samples gives the blood clearance of radioactivity and information on the incorporation into plasma proteins.

Fast liquid chromatography of samples taken can be used to determine the specific radioactivity of several low molecular weight compounds including both the given tracer and its main metabolic products (6). This, however, requires special equipment at site that was not availible for this study.

The external detection of ^{11}C is dependent on the type of detectors used. In this study single detectors were used because no alternative was available. Other detection techniques such as positron emission tomography should have allowed better determination of the radioactive concentration in different organs. Our experience is that minor movements gave only slight aberrations in the detection of radioactivity in the liver and the periferal tissue (muscle)

while the measurements from the stomach were more sensitive to detector positioning.

In this study the most relevant and reliable result was, however, that obtained from samples taken of blood, urine and exhaled air. This indicates the value of tracer studies with ^{11}C-compounds even without external detection facilities.

Homocysteine is closely linked with the metabolism of methionine. The amounts given were however small (about 0.5 mg, 4 nmol). A possible interaction was not evaluated in these studies. The measured parameters may also be affected by several physiological differences at the time of the investigations, such as diurnal rhythm or stress. The shape of the radioactivity curves i.e. the inital phase of the liver uptake, the radioactivity in the blood samples and the elimination by exhaled carbon dioxide, indicates, however, that the investigations were performed under similar conditions.

In the short time perspective the increase in energy intake did not give the expected improvement of the blood ammonia. However, several months later the blood ammonia had almost normalized. Futhermore the accelerated growth during this seven-month period as well as the recoverd clinical status are convincing signs of the improved nutrition and metabolism.

The two investigations with ^{11}C-methionine show that during the too low intake, relatively more of the tracer passed through the liver as low molecular weight fraction compounds in the blood plasma and reached the muscle. After the raised energy intake more of the radioactivity was retained in the liver. The transport compartment i.e. the low molecular weight fraction of blood plasma, contained less radioactivity and less radioactivity was detected over muscle tissue. As there was an unchanged elimination of radioactivity through exhaled air and urine the increased concentration in liver may reflect a more effective use of the labelled molecule.

The conclusions are based on two studies only, nevertheless the results coincide fairly well with the long-term observations of the patient. This encourages further studies in this application of tracer techniques using bio-molecules labelled with ^{11}C.

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