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[*N*-methyl-^{11}C]MeAIB, a Tracer for System A Amino Acid Transport: Preparation from [^{11}C]Methyl Triflate and HPLC Metabolite Analysis of Plasma Samples After Intravenous Administration in Man.

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Summary

MeAIB, α-methylamino-isobutyric acid, is an achiral synthetic amino acid which is a highly selective substrate for the A-type, or alanine-preferring, amino acid transport system. [*N*-methyl-^{11}C]MeAIB ([^{11}C]MeAIB) was prepared by reaction of [^{11}C]methyl triflate with AIB methyl ester, generated *in situ* from its corresponding hydrochloride, followed by hydrolysis of the ester function with aqueous NaOH. After HPLC-purification, the product was obtained in a 60-70% decay corrected yield counted from [^{11}C]methyl triflate. The total synthesis time was 32-37 min and the radiochemical purity of the product higher than 98%. HPLC analysis of plasma samples taken 5-30 min after the administration of [^{11}C]MeAIB to man showed that more than 95% of the total radioactivity in the plasma consisted of unchanged [^{11}C]MeAIB. The simple preparation and the high metabolic stability of [^{11}C]MeAIB makes this novel tracer a potential candidate for positron emission tomography investigations for the system A amino acid transport system *in vivo*.

Key words: [^{11}C]MeAIB, [^{11}C]methyl triflate, amino acid transport, metabolism, PET

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INTRODUCTION

The potential of positron emission tomography (PET) and amino acids labelled with short-lived radionuclides as a non-invasive method for the study of amino acid transport and protein synthesis in humans is well recognised (1). So far, studies have been hampered by the lack of suitable tracers that are specific for these two physiological processes. The labelled amino acid most commonly used in PET studies, [^{11}C]methionine, is not only a substrate for transport and protein synthesis but also for polyamine synthesis and transmethylation reactions.

System A is a hormonally regulated ubiquitous transport mechanism for neutral amino acids. It is energized and can actively pump substrates against a concentration gradient from plasma into cells. Insulin seems to stimulate system A activity *in vitro* and it would be interesting to study e.g. the effect of insulin resistance on this regulation mechanism *in vivo.* In addition, system A transport is increased in actively proliferating neoplastic tissues and system A selective radiopharmaceuticals may be useful for tumour studies by PET.

AIB (α-amino-isobutyric acid) is the prototype for system A selective amino acids (2). Several methods have been developed for the preparation of [^{11}C]AIB: [^{11}C]HCN has been used in the production of [1-^{11}C]AIB (3); [^{11}C]CH_3I and an azadisilolidine derivative of alanine (4) or the benzaldimine of alanine methyl ester (5) in the production of [3-^{11}C]AIB, and [^{11}C]acetone and the Strecker synthesis in the production of [2-^{11}C]AIB (6).

MeAIB, the *N*-methyl analogue of AIB concentrates in cells only via System A transport and has a very low metabolism (7). The higher specificity of MeAIB for the System A transport when compared with AIB favours this compound as a candidate for ^{11}C-labelling and use in PET studies for the System A transport. Three methods have been reported for the ^{11}C-labelling of MeAIB in different positions: [2-^{11}C]MeAIB has been prepared from [^{11}C]acetone using a Strecker synthesis (6). [*N*-methyl-^{11}C]MeAIB has been prepared from [^{11}C]formaldehyde (8), the use of [^{11}C]methyl iodide (9) or [^{11}C]methyl triflate is reported here.

The aim of this investigation was to find reliable and reproducible conditions for the production of [*N*-methyl-^{11}C]MeAIB from [^{11}C]methyl triflate, and to investigate the *in vivo* metabolism of this tracer by HPLC analysis of plasma samples after intravenous administration to man.

EXPERIMENTAL

Materials & General Methods

Materials

AIB and MeAIB were obtained from Aldrich. Other chemicals were obtained from commercial sources and were of analytical grade.

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Production of [^{11}C]methyl triflate

The [^{11}C]carbon dioxide was produced at the Accelerator Laboratory of Åbo Akademi with a 103 cm isochronous Efremov cyclotron using the $^{14}N(p,\alpha)\,^{11}C$ reaction. [^{11}C]Methyl triflate was prepared on line from [^{11}C]methyl iodide according to the procedures described in detail previously (10, 11).

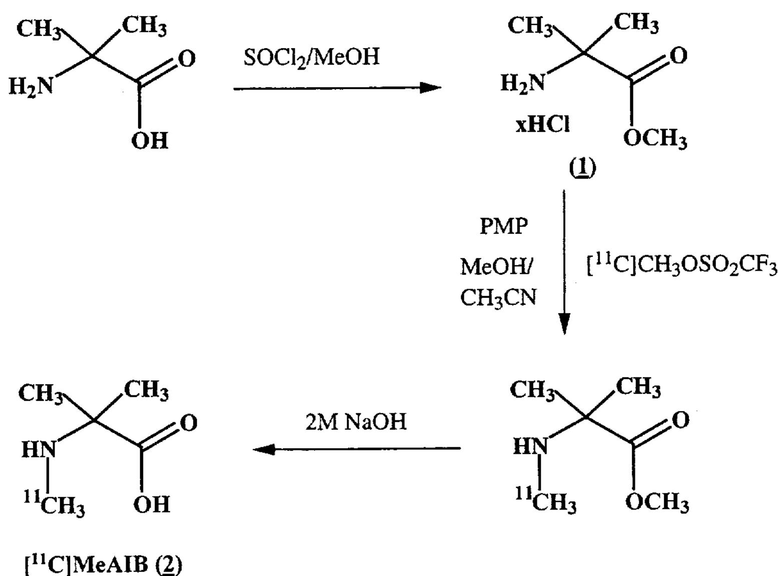

Figure 1. Synthesis of the precursor (1) and its use in the preparation of [^{11}C]MeAIB (2).

HPLC purification, formulation and analysis

Semi-preparative HPLC was performed using a strong anion exchange Phenomenex Selectosil SAX column (250 x 10 mm, 5 μm). UV-absorbance and radioactivity were monitored with a Pharmacia UV-1 UV-detector (wavelength = 254 nm) and a coaxial-type air ionization chamber (12) in series. Sterile propylene glucol/ethanol (7/3) and sterile physiological phosphate buffer (pH=7.4) were obtained from the Pharmacy of Turku University Central Hospital. Filters used in the sterile filtration of the final products were obtained from Gelman Ltd (Acrodisc, 0.22 μm). The radiochemical purity was analysed by HPLC using a Waters μ-Bondapak NH_2 column (300 x 3.9 mm, 10 μm) and a gradient program described previously (13).

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Preparation of AIB-OMe · HCl (1)

A stirred slurry of α-amino-isobutyric acid (AIB, 10.3 g, 100 mmol) in methanol (100 ml) was cooled to –10°C and thionyl chloride (8.8 ml, 120 mmol) was added dropwise for 90 min. The reaction temperature was increased to 50°C over 90 min, and the reaction then allowed to continue for 18 hours. After evaporation, the crude product was crystallized from methanol/ether giving 14.2 g (93%) of 1 as colourless crystals.

Preparation of [^{11}C]MeAIB (2)

[^{11}C]Methyl triflate was trapped at 0°C in a sealed reaction vessel containing 1 (1.0 mg) and 2,2,6,6,*N*-pentamethyl-piperidine (PMP, 0.8 μL) in 100 μL methanol/acetonitrile (2/1). The vessel was heated at 60°C for 1 min. Aqueous sodium hydroxide (2M, 60 μL) was added and the mixture heated for 3 min at 60°C. Mobile phase (300 μL) was added before injection onto the semi-preparative anion exchange HPLC column. [^{11}C]MeAIB eluted after 6-8 min with 2.5 mM NaH_2PO_4 in water/acetonitrile (17/83) and a flow of 8.0 mL/min (Fig. 2), with a retention time identical to a standard reference sample. Sterile propylene glycol/ethanol (7/3, 300 μL) was added to the eluent containing the purified product. After evaporation of the mobile phase, the residue was dissolved in 8 mL physiological phosphate buffer (pH=7.4) and filtered through a 0.22 μm sterile filter, giving a solution which was sterile and free from pyrogens.

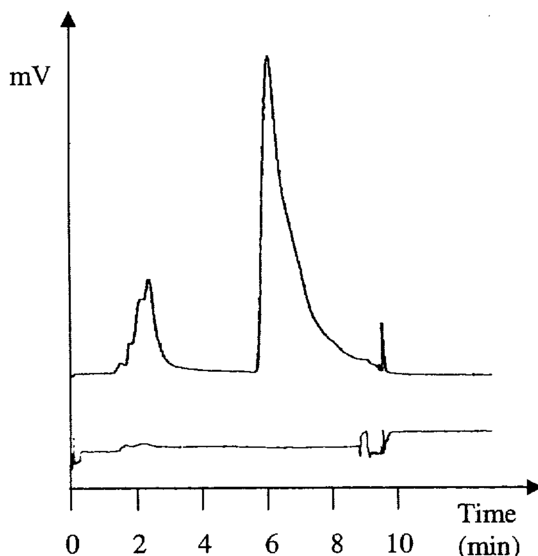

Figure 2. HPLC chromatograms from the purification of [*N*-methyl-^{11}C]Me-AIB. Upper: Radioactivity, lower: u.v. (change of HPLC eluent at 8 min)

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Analysis of plasma samples after intravenous administration to man

Sample preparation

Arteliazed venous blood (2 ml) derived from antecubital vein, arm heated by heating pad, was obtained at 5, 15, 20, 25 and 30 minutes after *i.v.* administration of [*N*-methyl-^{11}C]MeAIB. After centrifugation for 5 min at 3300 rpm, plasma (0.7 ml) was removed and mixed with aqueous 5-sulphosalicylic acid (10%, w/v; 0.7 ml). The mixture was centrifuged at 15000 rpm for three minutes and the supernatant was filtered through a Gelman Acrodisc LC13 PVDF 0.45 μm filter and used in the chromatographic analysis.

Chromatography

The liquid chromatograph consisted of a Merck L-7100 gradient pump system, a Rheodyne 7725i injector with a 1 ml loop, a Phenomenex Ultracarb ODS(20) column (10x250 mm, 7 μm), a Merck L-7400 UV-detector followed by a Packard Radiomatic 150TR detector with a 0.6 ml PET-cell. The data was processed by a Merck D-7000 HPLC System Manager.

Analysis programme 1

The column was eluted with a gradient programme of acetonitrile (B) in 0.05 M phosphoric acid. The programme started at 4 ml/min of 5% B; gradient from 4 ml/min of 5% B to 8 ml/min of 40% B between 4 and 6 minutes; back to 4 ml/min of 5% B between 9.5 and 10 minutes and end of run at 11 minutes. UV absorbance was measured at 225 nm.

Analysis programme 2

The column was eluted with a flow of 6 ml/min and a gradient programme of methanol (C) in an aqueous solution of sodium acetate (0.1M), citric acid (0.1M), EDTA (1mM), sodium heptane sulphonate (5 mM) and MeOH (5% v/v). The programme started at 0% C, gradient to 25% C between 3 and 8 minutes and gradient back to 0% C between 9 and 10 minutes and end of run at 10 minutes. UV absorbance was measured at 254 nm.

RESULTS AND DISCUSSION

Preparation of [^{11}C]MeAIB

The precursor for ^{11}C-labelling was prepared by a straightforward esterification procedure that afforded crystalline 1 in 93% yield. No visible degradation of 1, nor any change in [*N*-methyl-^{11}C]MeAIB yield have been observed during 5 years storage of 1 in a refrigerator. Preparation of ^{11}C-labelled MeAIB has attracted considerable interest during the last decade, the rationale being that

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MeAIB is even more selective than AIB for the A-type, or alanine-preferring, amino acid transport system. The molecular structure of MeAIB contains four structurally different carbons, which all can be subject to labelling with ^{11}C. The labelling of radiopharmaceuticals in different positions using ^{11}C has been shown to be a valuable tool for evaluation of their metabolism and subsequent optimisation of radioligand characteristics *in vivo* (14). In the case of MeAIB, the rate of metabolism in man is low and the labelling position less important. For the purpose of routine production of [^{11}C]MeAIB for PET, the labelling method which is most convenient when considering aspects such as unlabelled and labelled precursors can thus be chosen.

The most commonly used ^{11}C-labelled precursor has during the last two decades been [^{11}C]methyl iodide (15). Recently it has been shown that this precursor can in most syntheses be replaced by [^{11}C]methyl triflate, which offers several advantages, mainly due to its high reactivity (16). The synthesis of [*N*-methyl-^{11}C]MeAIB from [^{11}C]methyl iodide has been reported (9), the primary amino function in the precursor being protected as the BOC derivative to prevent formation of the *N,N*-di-methylated by-product. A 15 minutes hydrolysis with HI was found necessary for the removal of the BOC group.

In the preliminary evaluation of the synthesis of [*N*-methyl-^{11}C]MeAIB from 1, both [^{11}C]methyl iodide and [^{11}C]methyl triflate were evaluated. In both cases the amounts of 1, PMP and solvents were the same (see methods). It was found that using [^{11}C]methyl iodide and 5 min heating at 110°C gave 10-15% of product, [*N*-methyl-^{11}C]MeAIB methyl ester, whereas when using [^{11}C]methyl triflate and 1 min heating at 60°C, 70-80% of the product was obtained.

The subsequent hydrolysis of [*N*-methyl-^{11}C]MeAIB methyl ester to the product, [*N*-methyl-^{11}C]MeAIB, was found to proceed in more than 80% yield using 2M NaOH and 3 minutes heating at 60°C. In most routine productions, when high-specific radioactivity (3000-5000 mCi/μmol) [^{11}C]methyl triflate was used, only minute amounts (<0.1%) of the *N,N*-di-methylated product was found in the crude product. Occasionally, especially after change of target foil, the specific radioactivity of [^{11}C]methyl triflate has been much lower (100-500 mCi/μmol). In these cases up to 2-5% of di-methylated product was obtained, somewhat reducing the final yield of the product. This by-product was however well separated from the product on HPLC, which shows that, at least when working with high specific radioactivity ^{11}C, there is no need for protection of the primary amino function of the precursor 1.

Purification by HPLC (Figure 2) enabled separation from all labelled by-products, and gave a product with high radiochemical purity (>98%). The total isolated yield of [*N*-methyl-^{11}C]MeAIB was 60-70%, decay corrected from [^{11}C]methyl triflate, with a total synthesis time of 32-37 minutes.

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Analysis of plasma samples after intravenous administration to man

The metabolic stability of [N-methyl-^{11}C]MeAIB in humans was determined by HPLC analysis of plasma samples, withdrawn 5-30 minutes after administration. Plasma proteins were precipitated with 5-sulphosalicylic acid, previously used for [^{11}C]MHED (17), which we have found useful also for other hydrophilic compounds such as [^{11}C]DOPA and [^{11}C]MP4A. The recovery of radioactivity in the supernatant was higher than 95%.

The separation of small hydrophilic ^{11}C–labelled radiotracers from their metabolites using standard reversed-phase columns is poor, as these molecules elute close to the void volume. In analytical chemistry ion-exchange columns are often used in separation of this kind of compound. The equilibration of ion-exchange columns between analyses is a time-consuming process, especially when solvent gradients are used, and the use of these columns are thus normally not compatible with the short half-life of ^{11}C (20.3 min).

For separation of [N-methyl-^{11}C]MeAIB and labelled metabolites an Ultracarb HPLC column was used. This column brand has previously been used for [^{11}C]DOPA metabolite analysis (18), and been shown to give high retention of hydrophilic molecules. Analysis of the human plasma samples using two different solvent gradient systems detected in both cases only unchanged [N-methyl-^{11}C]MeAIB. An example of the results using analysis programme 2 is given in Figure 3. Taking the limitations in radioactivity detection sensitivity into account, the total amount of labelled metabolites, detected as minor peaks in the radiochromatograms, was no greater than 5%. There is thus no need for metabolite determinations in conjunction with [N-methyl-^{11}C]MeAIB PET studies, which simplifies the protocols for PET studies and the analysis of PET data.

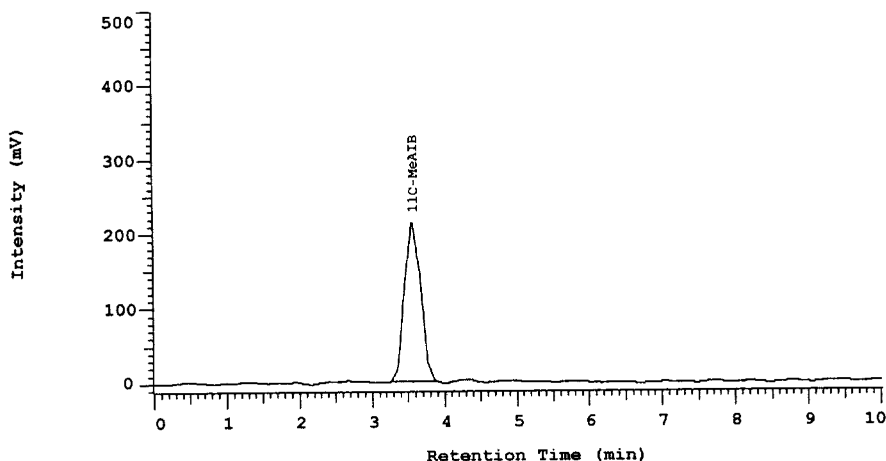

Figure 3. HPLC radiochromatogram from the analysis (Analysis programme 2) of a plasma sample taken 30 min after *i.v.* administration of [N-methyl-^{11}C]Me-AIB to humans.

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CONCLUSION

A simple and reliable method was developed for the production of [*N*-methyl-^{11}C]MeAIB, which is obtained in high yield and high radiochemical purity. HPLC metabolite analysis of human plasma samples demonstrated a high *in vivo* stability of [^{11}C]MeAIB. The simple preparation and the high metabolic stability of [^{11}C]MeAIB makes this novel tracer a potential candidate for positron emission tomography studies for the system A amino acid transport system *in vivo*.

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