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The Bücherer-Strecker synthesis of D- and L-(1-^{11}C)tyrosine and the in vivo study of L-(1-^{11}C)tyrosine in human brain using positron emission tomography

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Abstract. The synthesis of D- and L-(1-^{11}C)tyrosine, starting with ^{11}C-cyanide, is reported. DL-(1-^{11}C)Tyrosine was prepared by the Bücherer-Strecker reaction, from carrier added ^{11}C-cyanide with an incorporation of 80% in 20 min. The isolation of the pure D- and L-amino acid isomers from the enantiomeric mixture was accomplished within 15 min by preparative HPLC using a chiral stationary phase and a phosphate buffer as the mobile phase. Typically, the total synthesis time was 50 min (including purification) from end of trapping of ^{11}C-cyanide, with a radiochemical yield of D- and L-amino acid of 40%–60%. The D- and L-(1-^{11}C)tyrosine were both obtained optically pure, with a carrier added specific activity of 0.3–0.5 Ci/mmol and a radiochemical purity better than 99%. The ^{11}C labelled L-tyrosine was used in an in vivo study in the human brain using positron emission tomography (PET).

Key words: ^{11}C-Tyrosine – D-Tyrosine – L-Tyrosine – (1-^{11}C)Tyrosine – Positron emission tomography

Compounds labelled with short lived radionuclides have been used for studying human metabolism and neuroreceptor characteristics in vivo by use of positron emission tomography. Amino acids labelled with ^{11}C, ^{13}N and ^{18}F are potentially useful tracers for the in vivo assessment of amino acid uptake and metabolism. Emphasis has been placed on the possibility of synthesizing amino acids labelled with ^{11}C in various positions and in the enantiomerically pure form (Halldin 1984; Kilbourn 1985).

The Bücherer-Strecker synthesis has been used in the preparation of racemic ^{11}C-amino acids, labelled in the carboxylic position (Hayes et al. 1976) or in the 2 position (Halldin and Långström 1985). The reaction of ^{11}C-carbon dioxide with the organometallic α-lithioisocyanide reagent has been utilized in the synthesis of several racemic amino acids (e.g., tyrosine) (Bolster et al. 1986) labelled in the carboxylic position.

Separation of the L-enantiomer from the ^{11}C labelled amino acid racemate is necessary since physiological studies of cerebral protein synthesis and transport of amino acids across membranes usually require the L-amino acid. The racemic mixture can be resolved by oxidative deamination (Casey et al. 1981; Antoni and Långstrom 1987), liquid chromatography separation employing a chiral mobile phase (Washburn et al. 1982) or by using human serum albumin coupled to a Sepharose resin (Wu et al. 1981). DL-(1-^{11}C)-DOPA and DL-(1-^{11}C)-tyrosine have been resolved using a chiral stationary phase consisting of a polymer bound (L-proline)-Cu complex (Bolster et al. 1983, 1986).

We report (Fig. 1) the synthesis of racemic DL-(1-^{11}C)tyrosine (I) by the Bücherer-Strecker approach using ^{11}C-cyanide and the resolution of the racemic mixture by preparative HPLC yielding enantiomerically pure D- and L-(1-^{11}C)tyrosine (II, III). Resolution of the L-isomer from the racemic mixture by oxidative deamination with immobilized D-amino oxidase was also investigated.

PET studies with ^{11}C labelled DL-tyrosine have shown that this labelled amino acid may be used in the detection of human tumors (Bolster et al. 1986). Decreased L-tyrosine transport across the fibroblast membrane in schizophrenic patients has been observed in an in vitro study by Bjerkenstedt et al. (1986), which may have implications for the study of the pathophysiological mechanisms and genetics of schizophrenia and for designing new therapeutic approaches. In vivo studies of the transport of L-(1-^{11}C)tyrosine in normal volunteers and in schizophrenic patients have now been undertaken using PET. In this paper, the distribution of ^{11}C labelled L-tyrosine in normal human brain is presented.

Materials and methods

General. The ^{11}C-carbon dioxide was produced at the Karolinska Hospital with a Scanditronix RNP 16 cyclotron using 16 MeV protons in the ^{14}N(ρ,α)^{11}C reaction. The gas target was irradiated for 60 min with a beam intensity of 40–45 μA. The ^{11}C labelled cyanide was produced from ^{11}C-carbon dioxide in a two step on line conversion (Christman et al. 1975) and trapped directly into the reaction solution. The Bücherer-Strecker reaction was performed in a stainless steel reaction vessel (Halldin and Långström 1985). Potassium cyanide was obtained from Merck and ammonium carbonate from Analar (Hopkin & Williams, LTD). The ion retardation resin, AG11A8 (50–100 mesh) was obtained from BioRad. The nonradioactive starting material p-hydroxyphenylacetaldehyde, was not commercially available and was therefore synthesized by a literature method (Robbins 1966) and used as the bisulphite adduct. Marfey's reagent (Marfey 1984) was purchased from Pierce. Radiochemical purity was analyzed by

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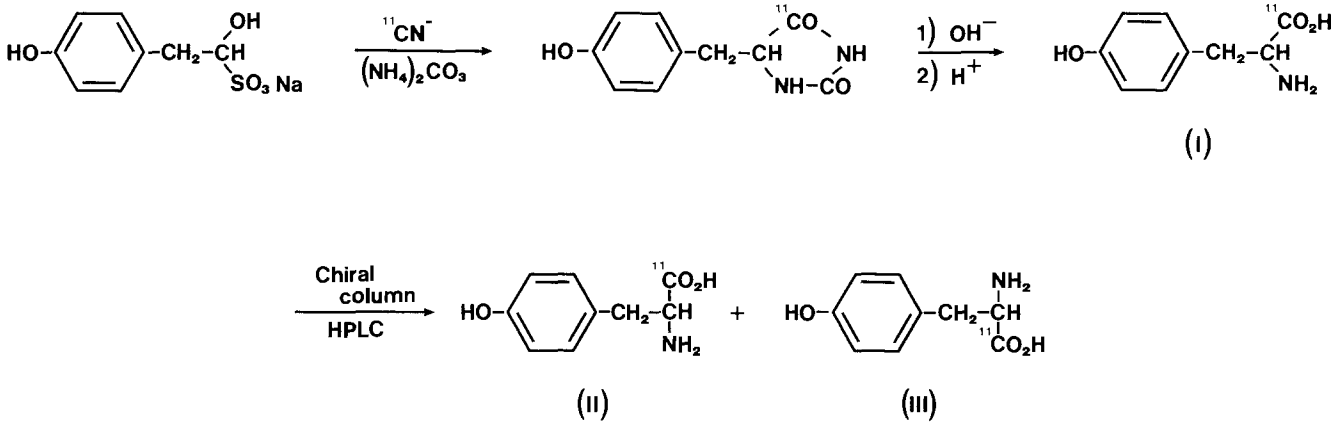

Fig. 1. Reaction scheme for the synthesis of D- and L-(1-^{11}C)tyrosine

HPLC using a Constametric III pump and an ERC-7510 UV spectrophotometer equipped with a μ-Bondapak-NH_2 column (Waters, 10 μm, 300 × 3.9 mm) in series with a Berthold LB 503 detector. The mobile phase was acetonitrile and potassium dihydrogen phosphate (0.01 M, pH = 3) in a ratio of 85:15. The flow was 2.0 cm^3/min and the wavelength was 254 nm. A Novapak C18 column (Waters, 5 μm, 150 × 3.9 mm) was used for analysis of the optical purity. The mobile phase was acetonitrile and triethylammonium phosphate (0.05 M, pH = 3) in a ratio of 23.5:76.5. The flow rate was 1.5 cm^3/min and the wavelength was 340 nm. The preparative HPLC column used for optical resolution was CHIRALPAK WH, 250 × 10.0 mm purchased from Daicel Chemical Industries. The flow rate was 6.0 cm^3/min, column temperature 50° C and the wavelength was 254 nm. The mobile phase was 0.1 M sodium dihydrogen phosphate (pH 4.5).

DL-(1-^{11}C)*Tyrosine (I).* (Fig. 1) The ^{11}C labelled cyanide was trapped directly in the stainless steel reaction vessel containing 0.005 M sodium hydroxide (1.0 cm^3), potassium cyanide (8 mg, 0.12 mmol), ammonium carbonate (60 mg, 0.63 mmol) and p-hydroxyphenylacetaldehyde bisulphite adduct (80 mg, 0.33 mmol). The vessel was closed and heated at 170° C for 10 min. The vessel was cooled and 10 M sodium hydroxide (0.5 cm^3) was added. The vessel was closed and reheated to 170° C for 10 min. After cooling, concentrated acetic acid (0.5 cm^3) was added (to pH 8.0 ± 0.3). The final solution was eluted through an ion retardation column (AG11A8, 5.0 × 1.2 cm) with 3 × 2.0 cm^3 water.

D- *and* L-(1-^{11}C)*Tyrosine (II, III).* (Fig. 1) Phosphoric acid (1 M, 1.0 cm^3) was added to the eluate from the ion retardation column prior to evaporation on a rotary evaporator. The residue was redissolved in 1.0 M phosphoric acid (150 μl) and 0.1 M sodium dihydrogen phosphate buffer (pH 4.5, 850 μl). For optical resolution the sample was injected into the Chiralpak column containing the chiral stationary phase and 0.1 M sodium dihydrogen phosphate buffer (pH 4.5) as eluent. D-(1-^{11}C)Tyrosine eluted after 6.5–8 min and L-(1-^{11}C)tyrosine eluted after 13–15 min with the same retention times as standard reference samples (Fig. 2). The appropriate LC fraction was pH adjusted and sterilized by passage through a Millipore (0.22 μm) filter prior to use in the PET investigation.

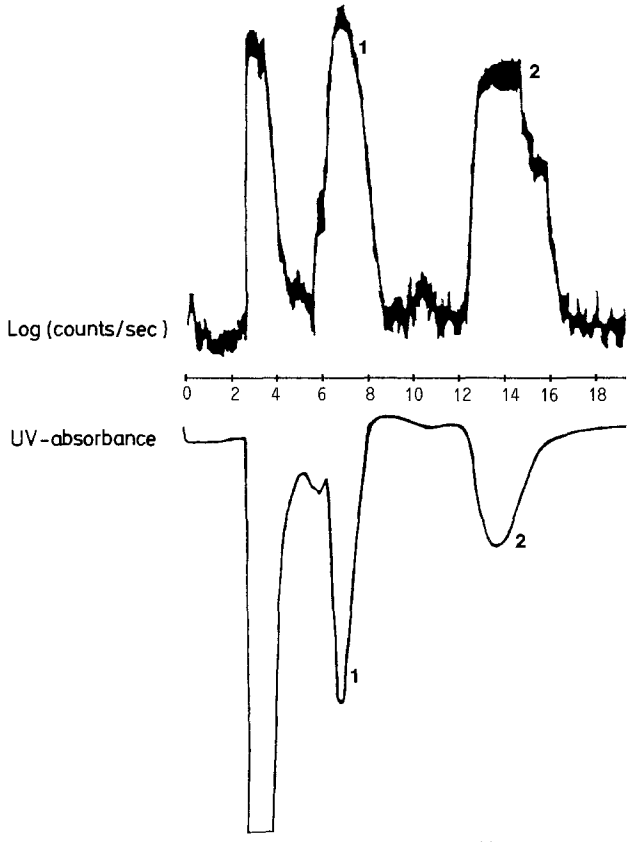

Fig. 2. HPLC chromatogram of D- and L-(1-^{11}C)tyrosine (log-(counts/s) and UV-absorbance). *1* = D-tyrosine and *2* = L-tyrosine

Determination of optical purity of D- *and* L-(1-^{11}C)*tyrosine by Marfey's reagent.* An aliquot (100 μl) of the ^{11}C labelled amino acid obtained above was placed in a 1.0 cm^3 reaction vial. Marfey's reagent (FDAA) (1% in acetone, 200 μl) and 1.0 M sodium bicarbonate solution (40 μl) were added and the mixture was heated at 60° C for 10 min. After cooling, 2.0 M hydrochloric acid (20 μl) was added. When the evolution of gas had ceased, the sample was analyzed by HPLC. The derivatives of L- and D-(1-^{11}C)tyrosine eluted after 5.4 and 8.2 min, respectively (same retention times as standard reference samples).

PET investigation. The PET investigation was performed on a 27-year-old healthy male volunteer. L-(1-^{11}C)Tyrosine

(325 MBq) was injected i.v. as a bolus in an antecubital vein. An arterial cannula was inserted in the contralateral arm for blood sampling. A 4 ring Scanditronix AB PET system (PC-384) with 96 BGO detectors per ring was used giving 7 transverse sections. Quantitative data on the regional uptake of the ^{11}C labelled L-amino acid was obtained by sequential PET scans for 24 min (corrected for ^{11}C decay). The spatial resolution of the reconstructed images is 7.6 mm (full width at half maximum). Computed tomography scans were also performed to enable the anatomical localization of the uptake of the tracer (Bergström et al. 1981).

Results and discussion

Chemistry

The nonradioactive precursor, p-hydroxyphenylacetaldehyde-bisulphite adduct, was obtained in improved yields by a modification of a literature method (Robbins 1966). By allowing the temperature of the solution to rise to only 110° C instead of 120° C, the total chemical yield was increased from 11% to 30%. Above 110° C the solution changed from colorless to dark brown, which was assumed to be an indication of decomposition since yields were drastically reduced at higher temperature.

The Bücherer-Strecker synthesis is almost always performed under carrier added conditions to increase the radiolabelling yields. In our investigation the incorporation of ^{11}C-cyanide was found to be reproducible in carrier added experiments (between 78–82%). In no carrier added experiments a conversion of only 2–4% was achieved. As has been reported previously (Washburn et al. 1979; Zalutsky et al. 1981), yields of the ^{11}C-hydantoin were found to be dependent on the reaction temperature with incorporation of ^{11}C-cyanide increasing from 30–35% at 120° C to 80% at 170° C. No significant increase in yield was obtained with reaction times greater than 10 min in either the hydantoin formation step or in the hydrolysis. The mixture containing the D,L-amino acid was eluted through an ion retardation column (Bio-Rad AG11A8) to decrease the salt concentration. After evaporation of the eluate, phosphoric acid was added to the residue along with sodium dihydrogen phosphate to ensure a homogenous solution prior to injection on the HPLC column.

Since the Bücherer-Strecker synthesis always yields a racemic mixture of L- and D-amino acids, it is usually considered desirable to separate the two enantiomeric forms before use in in vivo imaging of physiological processes. The biosynthetic resolution with immobilized D-amino oxidase yields the L-amino acid and alpha-keto acid (which is subsequently separated by ion chromatography) and has been successfully used in the preparation of a number of ^{11}C-amino acids. In our experience, the enzyme method worked satisfactorily at first but the enzyme activity successively decreased. The chiral column HPLC separation of the D- and L-isomers was, in our case, the method of choice. This method is not only reproducible but also enables the isolation of both the D- and L-amino acid from the racemic mixture.

The total synthesis time was 50 min (including purification) from end of trapping of ^{11}C-cyanide, with a radiochemical yield of D- and L-tyrosine of 40%–60%. Specific activities of the carrier added D- and L-amino acid were 0.3–0.5 Cl/mmol. The D- and L-amino acids were obtained optically pure (optical purity was measured both with the chiral column and with Marfey's reagent) and with a radiochemical purity better than 99%.

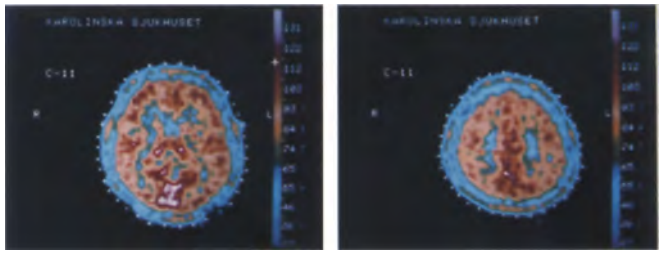

Fig. 3. PET scans showing radioactivity accumulated (corrected for decay) during 24 min after i.v. injection of L-(1-^{11}C)tyrosine (325 MBq). *Left:* The slice shown is through the striatum (3 mm above Monroe's foramen), the thalamus and the occipital cortex and is parallel to the canto meatal plane. *Right:* The slice shown is 27 mm above the first slice and is through the prefrontal cortex, the cingular and the occipital cortex. The tracer concentration ranged between 123–149 nCi/ml and 120–157 nCi/ml for the left and right slices, respectively, during the investigation

PET investigation

The regional uptake of radioactivity studied in vivo in the human brain following the injection of L-(1-^{11}C)tyrosine is shown in Fig. 3. The radiotracer crossed the BBB and a regional accumulation was observed in the cortical and subcortical structures, especially in the occipital cortex, limbic cortex, and the thalamus.

L-Tyrosine shares an uptake site for neutral amino acids (the L-system) at the BBB (Pardridge and Oldendorf 1977; Hardebo and Owman 1980). In vitro studies of the fibroblast membrane demonstrated a decreased transport of tyrosine in schizophrenic patients (Bjerkenstedt et al. 1986). Such a disturbance in the transport of L-tyrosine across the BBB may be expected to have serious consequences for the synthesis of neurotransmitters and proteins in the brain. L-(1-^{11}C)Tyrosine will be useful as a tracer for the determination of the influx rate of L-tyrosine in schizophrenic patients and healthy volunteers.

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