ENZYMATIC SYNTHESIS OF [^{11}C]*N*-ACETYLSEROTONIN

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SUMMARY

An enzymatic synthesis of [^{11}C] *N*-acetylserotonin is described. [1-^{11}C] acetate is produced by reacting $^{11}CO_2$ with methylmagnesium bromide and converted to [^{11}C]acetylCoA by passage over a first enzyme reactor containing immobilized acetylcoenzyme A synthetase. The produced [^{11}C]-acetylCoA is converted to [^{11}C]*N*-acetylserotonin with a second enzyme reactor containing immobilized acetylcoenzyme A : arylamine *N*-acetyltransferase. The labelled end product is purified by means of ionexchange chromatography and is obtained in a solution suitable for intravenous injection.

After irradiation with 18 MeV protons at 15 µA for 20 min, 25 mCi with a specific activity of 250 mCi/µmol were obtained. The whole synthesis starting from EOB takes 40 min.

Key Words : Carbon-11 *N*-acetylserotonin, enzymatic synthesis, receptor ligand.

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INTRODUCTION

N-acetylserotonin (*N*-acetyl-5-hydroxytryptamine) is an endogenous substance, secreted by the pineal gland and other brain regions. Besides being a precursor for the melatonin synthesis, *N*-acetylserotonin is also a biological active agent. This was recently demonstrated by Psarakis et al., who found an interaction of ^{3}H-labelled *N*-acetylserotonin with specific receptors, producing analgesia. For this effect an acetyl group on the terminal amine function and a free hydroxyl group on C5 of the indole skelet were required (1).

To enable us to confirm and extend these preliminary findings with in vivo experiments, the synthesis of ^{11}C-labelled *N*-acetylserotonin was elaborated. Labelling with the positron emitter carbon-11 ($T_{\frac{1}{2}}$ 20.4 min) will allow us to perform in vivo biomedical studies with this compound using Positron Emission Tomography (PET). [^{11}C] *N*-acetylserotonin can be obtained starting from serotonin and the available [^{11}C] acetylcoenzyme A (2), using the enzyme arylamine acetyltransferase (acetylcoenzyme A : arylamine *N*-acetyltransferase ; EC 2.3.1.5) (Fig. 1).

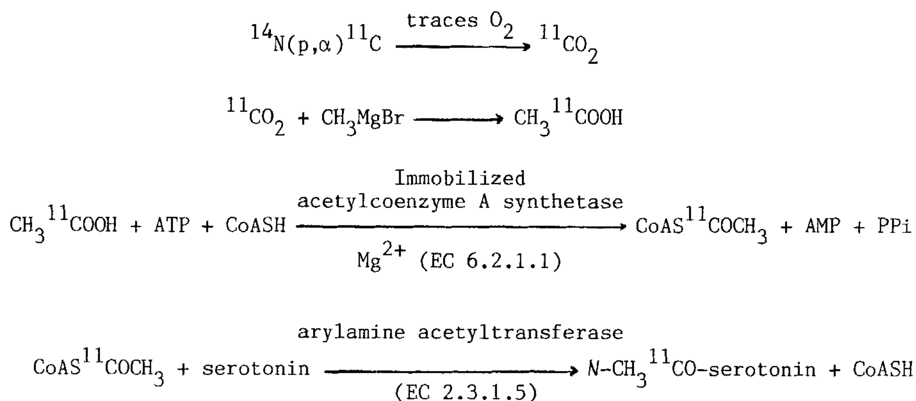

Fig.1. Scheme for the synthesis of [^{11}C]*N*-acetylserotonin

EXPERIMENTAL

Materials

Acetylcoenzyme A synthetase (ACS) from baker's yeast, trilithium coenzyme A (CoA), disodium adenosine-5'-triphosphate (ATP), trilithium acetylcoenzyme A, 5-hydroxytryptamine HCl (serotonin HCl) and *N*-acetyl-5-hydroxytryptamine (*N*-acetylserotonin) were purchased from Sigma Chemical Company (USA). Other reagents were of analytical grade from Janssen Chimica (Belgium) or Merck (FRG). p-Chloromercuribenzoate immobilized on agarose beads was obtained from Pierce. DEAE silica (20-40 µm particle size, 100 Å pore size) and controlled pore glass beads (CPG 500, 125-180 µm ; CPG 240, 75-125 µm) were from Serva Feinbiochemica GmbH. The ionexchange resins Bio-Rex 70 (200-400 mesh) and Bio-Rex 5 (50-100 mesh) were from Bio-Rad Laboratories. The enzyme arylamine acetyltransferase was purified from pigeon liver.

The [1-^{11}C] acetate and [^{11}C] acetylCoA production, the acetylcoenzyme A synthetase immobilization on CNBr activated CPG 500 glass beads and related enzyme reactor preparation were as previously described (2).

Arylamine acetyltransferase

The pigeon liver enzyme is isolated and purified using a combination of ammonium sulphate precipitation and affinity chromatography on an immobilized enzyme inhibitor. Details concerning this purification procedure are given elsewhere (3).

The enzyme in solution and the immobilized enzyme were assayed spectrophotometrically as described by Tabor et al. (4). The incubation mixture contained in a final volume of 750 µl : 50 µmol potassium phosphate pH 8.0, 0.10 µmol p-nitroaniline, 0.05 µmol CoASAc, 5.0 µmol EDTA and 1.0

μmol dithiotreitol. The reaction was started by adding 50 μl enzyme solution (0-70 mU). The decrease in absorbance at 405 nm was followed at room temperature. For assaying the immobilized enzyme in a batch procedure, the same reaction mixture was pipetted onto 20 mg enzyme-loaded glass beads. The mixture was gently swirled and the absorbance of the supernatant was measured from time to time after the glass beads were allowed to settle.

One unit is defined as that amount of enzyme which catalyses the *N*-acetylation of one μmol p-nitroaniline per min using the conditions described.

Preparation of the arylamine acetyltransferase reactor

One gram CPG 240 (75-125 μm) was derivatized with γ-aminopropyl silane and glutaraldehyde according to the method of Weetall (5).

The glass beads were thoroughly washed to remove excess of glutaraldehyde and stored at 4 °C under water.

Before use, the derivatized glass beads were divided in 100 mg portions and 1.0-1.2 units arylamine acetyltransferase dissolved in 100 μl preservative buffer (0.1 M potassium phosphate pH 7.0 containing 1 mM dithiotreitol and 1 mM EDTA) are added at 0-4 °C in the presence of 100 μl 40 mM $NaCNBH_3$ dissolved in 0.3 M potassium phosphate pH 7.5.

After 1 h of gently swirling, the enzyme-loaded glass beads were washed with preservative buffer and 1 ml 0.2 M $NH_2OH.HCl/KOH$ pH 7.2 containing 2 mM dithiotreitol and 20 mM $NaCNBH_3$ was added to block the remaining reactive end-groups. After 1 h of shaking at 0°-4° C the glass beads were thoroughly washed with preservative buffer and packed in a glass column (9.8 x 0.5 cm i.d.). The reactor was stored aseptically at 0-4 °C under preservative buffer.

Set-up of the [^{11}C]N-acetylserotonin production (Fig. 2)

The set-up for the subsequent $^{11}CO_2$, [1-^{11}C]acetate and [^{11}C]acetylCoA production was identical to the one used for the [^{11}C]N-acetyl-D-glucosamine production (6). To purify [^{11}C] acetylCoA, it was loaded on a DEAE Si column (H). Elution was started at 2.0 ml/min with 0.2 M glycine/KOH pH 9.5 containing 1.5 M NaCl. The fraction containing [^{11}C]-acetylCoA (1.1 ml), was collected in a vial (I) containing 50 µl 70 mM serotonin HCl.

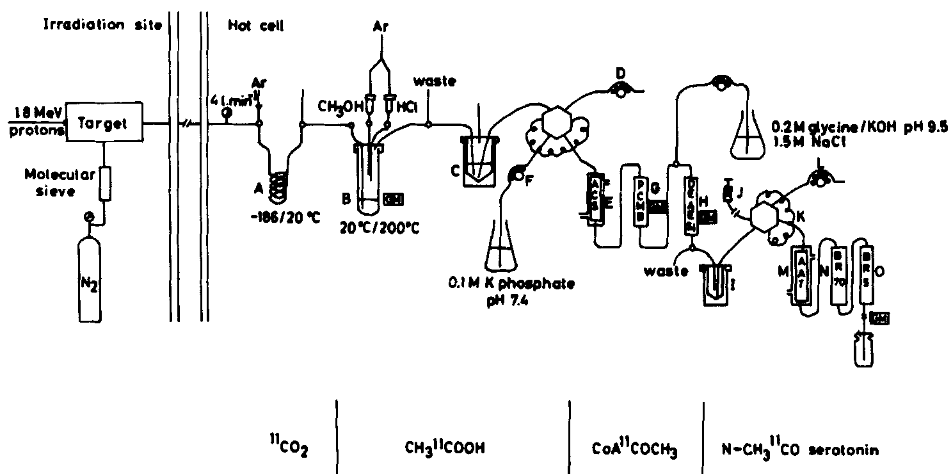

Fig.2. Set-up for the synthesis of [^{11}C]N-acetylserotonin.

To adjust the pH to 9.5, 80 µmol KOH were added using a syringe (J). By means of the same syringe, the content of the vial was mixed and loaded into a sample loop (K : 23 x 0.24 cm i.d.). Using a peristaltic pump (L), [^{11}C]acetylCoA cofactor mixture was sent at 1.0 ml/min and at 35 °C over the arylamine acetyltransferase reactor (M) with 25 mM potassium phosphate pH 8.0.

The produced [^{11}C]N-acetylserotonin is purified on-line using a cation- and anionexchanger, equilibrated with 0.05 M sodium phosphate pH 7.4. The first column (N ; Bio-

Rex 70, 4.8 x 0.49 cm i.d.) retains the excess of serotonin and the following column (O ; Bio-Rex 5, 2.4 x 0.55 cm i.d.) retains traces of unreacted [^{11}C] acetylCoA. [^{11}C] *N*-acetyl-serotonin is not retarded and elutes in 7 ml with a final pH of 6.9. Radioactivity was monitored with G.M. tubes.

Conditioning of the columns

The acetylcoenzyme A synthetase reactor, the p-chloro-mercuribenzoate gel and the DEAE Si100 column are equilibrated, regenerated and stored as previously described (6). Before use, the arylamine acetyltransferase reactor is equilibrated with 25 mM potassium phosphate pH 8.0 and placed in a water jacket at 35 °C. After the synthesis, the enzyme reactor is stored under preservative buffer at 0-4 °C.

The Bio-Rex 70 column is rinsed with 0.05 M sodium phosphate pH 7.4 and stored under the same buffer. The Bio-Rex 5 column is regenerated with 1 M HCl and stored under 0.05 M sodium phosphate pH 7.4.

RESULTS AND DISCUSSION

[^{11}C] *N*-acetylserotonin production

[^{11}C] acetyl CoA is converted in the presence of serotonin to [^{11}C] *N*-acetylserotonin using the enzyme acetylcoenzyme A : arylamine *N*-acetyltransferase (EC 2.3.1.5) as catalyst. Pigeon liver was selected as the enzyme source because of the high affinity of this enzyme for acetylcoenzyme A (7).

Details concerning the enzyme immobilization, reactor design and the optimal reaction conditions for the *N*-acetylation of serotonin are given elsewhere (8). Using the reactor prepared as described in the experimental section, up to 70 % conversion of [^{11}C] acetylcoenzyme A into [^{11}C] *N*-acetyl-serotonin can be obtained.

On-line purification of [^{11}C]*N*-acetylserotonin

The excess of the substrate serotonin leaving the arylamine *N*-acetyltransferase reactor has to be eliminated because of its possible interfering biological effects. The terminal amine function (pK_a 9.8) can easily be protonated, allowing retention on a cationexchanger. For this purpose, a cationexchanger based on a styrene divinylbenzene matrix (AG 50 W) was not useful because this resin exhibits a strong non-specific adsorption of aromatic compounds (serotonin, *N*-acetylserotonin and acetylcoenzyme A). Although very well separated from serotonin, a broad spreading of the labelled end-product was obtained. Better elution profiles were achieved with a weakly acidic cationexchanger based on an acrylic polymer (Bio-Rex 70).

For the same reason, Bio-Rex 5 (an intermediate base anionexchanger based on a polyalkyleneamine lattice) was used to eliminate traces of unreacted [^{11}C] acetylcoenzyme A. Use of the Bio-Rex 70 and Bio-Rex 5 columns, resulted in a labelled end-product free of serotonin and [^{11}C]-acetylcoenzyme A.

Radiochemical purity and specific activity

The labelled end-product was submitted to radiochromatography by HPLC using the conditions previously described (3). 25 µl were injected onto a Spherisorb 5 ODS 2 (150 x 3.2 mm i.d.) and eluted at 1 ml/min with 0.1 M sodium acetate pH 4.75/methanol (75/25 ; v/v). Simultaneous uv detection at 275 nm and radioactivity detection (NaI/Tl) were applied. A single radioactive peak coeluting with the *N*-acetylserotonin uv peak at 5.3 min was obtained. Figure 3 shows that the produced [^{11}C] *N*-acetylserotonin is radiochemically pure.

After irradiating with 18 MeV protons for 20 min at 15 µA, 25 mCi [^{11}C]*N*-acetylserotonin with a specific activity of 250 mCi/µmol were obtained at the end of the synthesis (EOB + 40 min). The carrier amount was directly determined

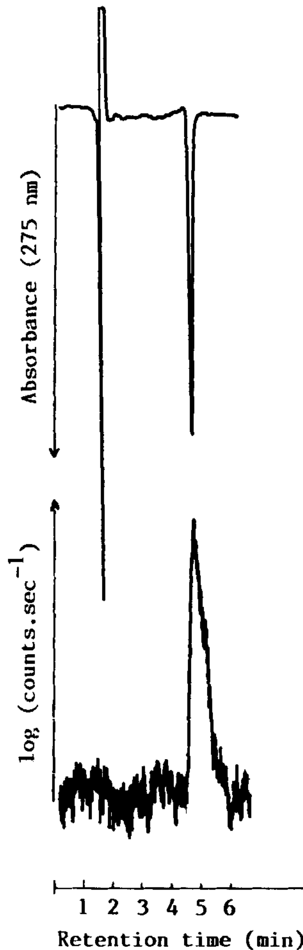

Fig. 3. Radiochromatography of the purified [^{11}C] *N*-acetyl serotonin.

from the uv chromatography, using a *N*-acetylserotonin standard curve. From the radioactivity measured in the collection flask and the different parts of the set-up, one can calculate that 25 % of $^{11}CO_2$ is incorporated into [^{11}C]*N*-acetylserotonin.

CONCLUSION

The receptor ligand, N-acetylserotonin was labelled with carbon-11 using a specific enzyme catalyzed reaction. The labelled compound was obtained in a sufficient radioactivity amount and specific activity to allow in vivo biomedical studies with PET.

This paper once more illustrates the applicability of [^{11}C]acetylcoenzyme A as a precursor (2). Using the same strategy, carbon-11 labelling of other interesting biogenic amines can be elaborated.

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