SYNTHESIS OF γ-AMINO[4-^{11}C]BUTYRIC ACID (GABA)

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Summary

A one-pot synthesis of no-carrier added γ-amino[4-^{11}C]butyric acid (GABA) starting with hydrogen [^{11}C]cyanide prepared from [^{11}C]carbon dioxide, is presented. Hydrogen [^{11}C]cyanide was trapped in tetrahydrofuran/potassium hydroxide in the presence of the amino polyether Kryptofix 2.2.2. A Michael addition with ethyl acrylate followed by a selective reduction and hydrolysis of the resulting amino ester gave [4-^{11}C]GABA. The radiochemical purity of GABA was higher than 99 % and the decay corrected radiochemical yield was 60-65% based on the amount of H[^{11}C]CN used. The total synthesis time including purification was around 40 min, counted from the start of the Michael addition reaction.

Key words: ^{11}C-GABA, γ-amino[4-^{11}C]butyric acid

Introduction

The so called fast transmitters, either excitatory neurotransmitting amino acids such as glutamate and aspartate or the inhibitory γ-aminobutyric acid (GABA), are involved in a multitude of important regulatory processes in the brain. Alterations of the GABA levels in the central nervous system are found in several psychiatric disorders such as epilepsy,[1] Huntington's chorea,[2] Schizophrenia[3] and Alzheimer's[4] disease. Although the mechanism of the involvement of GABA is unknown, loss of GABAergic neurons and decreased GABA concentrations in the brain may indicate its physiological role and connection with these diseases.

GABA has the lowest brain uptake index of all the putative central neurotransmitter substances[5a,b] partly depending on its low solubility in lipids which prevents passive diffusion through the blood brain barrier (BBB). Whether the restricted permeation of GABA into the brain also involves an enzymatic barrier situated in the endothelial cells is not yet known and can not be ruled out as a possible mechanism. There may, however exist a low affinity transport system of GABA into the brain.[6] The biosynthesis of GABA is mainly via

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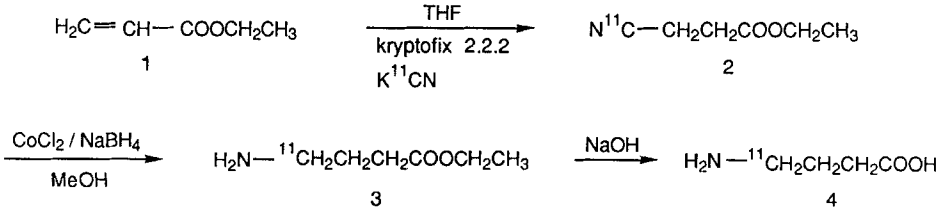
glutamate decarboxylation although oxidative degradation of putrecine may contribute.[7] Positron emission tomography (PET) studies using [^{11}C]GABA combined with other labelled tracers e.g. glutamate may give the opportunity to study the pathophysiology of the above mentioned diseases and to determine whether the postulated mediated transport involves any of the known carriers participating in the transport of amino acids through the BBB.

GABA has earlier been synthesized labelled with ^{13}N using an enzymatic reaction.[8] The longer half-life of ^{11}C enables extended investigation times compared with ^{13}N, so we have therefore developed a one-pot synthesis of no-carrier added (n.c.a) [4-^{11}C]GABA starting with hydrogen [^{11}C]cyanide. The labelled HCN was prepared from [^{11}C]carbon dioxide according to a well established procedure.[9] The hydrogen [^{11}C]cyanide obtained was trapped in tetrahydrofuran/potassium hydroxide in the presence of the amino polyether Kryptofix 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazobicyclo-8,8,8-hexacosan). A Michael addition with ethyl acrylate followed by a selective reduction and hydrolysis of the resulting amino ester gave [4-^{11}C]GABA according to Scheme 1.



Scheme 1

Results and discussions

The Michael addition reaction was found to be mostly dependent on the temperature and water concentration and rather insensitive to the amount of substrate *1* at the concentration level used (0.46-0.65 mmol). Reaction temperatures above 90°C resulted in the formation of unidentified lipophilic by-products. Increased water concentration (>5%) decreased the rate of the reaction. At the temperature used the Michael addition reaction was complete within 5 min giving the labelled nitrile *2* in 70-95 % radiochemical yield with unreacted [^{11}C]cyanide as the second major product. The selective reduction of the nitrile was carried out using sodium

borohydride and cobaltous chloride. The nitrile was activated towards the reducing agent by complexation with cobalt boride (Co_2B) formed *in situ*.[10] After 5 min reaction time at 50°C compound 3 was obtained and only traces of the nitrile could be detected by HPLC analysis. The following alkaline hydrolysis converted the amino ester to GABA 4 in high yield. Acidic hydrolysis using 6 M hydrochloric acid was tested but the reaction was found to be slower and produced more by-products. Following purification [4-^{11}C]GABA was obtained in 60-65% radiochemical yield, decay corrected and with a radiochemical purity higher than 99%. The specific activity of the labelled GABA was in the order of 4 GBq/μmol. The total synthesis time was around 40 min including purification, counted from start of the Michael addition reaction. The remaining amount of cobalt after purification was determined by atomic absorption to be less than 0.4 μg.

Another reaction route was originally worked out based on a substitution of ethyl 3-bromo- or 3-iodopropanoate. This route gave high yields of the nitrile in cold synthesis, however, attempts to perform labelling syntheses failed and only unreacted H[^{11}C]CN was obtained. A plausible explanation for this may be that the mechanism of this substitution involves an elimination addition sequence where the cyanide acts initially as a base, promoting the elimination followed by a Michael addition.[11] In synthesis with trace amounts of cyanide, as the case is with n.c.a [^{11}C]cyanide, the concentration of the elimination product (ethyl acrylate) will necessarily be very low i.e. in the same order of concentration as the cyanide. Consequently, very little Michael addition of cyanide with ethyl acrylate will occurr.

Material and Methods

General.

The ^{11}C was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using the tandem van de Graaf accelerator at the University of Uppsala. The [^{11}C]carbon dioxide produced was trapped in a lead-shielded oven containing 4-Å molecular sieves and transported to the chemistry laboratory. Hydrogen [^{11}C]cyanide was prepared by reducing [^{11}C]carbon dioxide to [^{11}C]methane using H_2 and a Ni catalyst at 400°C followed by reaction with NH_3 at 1000°C catalysed by Pt.[9] Analytical LC was performed on a Hewlett-Packard 1090 liquid chromatograph equipped either with a 250x4.6 mm (i.d.), Alltech C-18 reversed phase 10 micron column (A) or a 250x4.6 mm (i.d), Nucleosil LC-NH_2 5 micron column (B) in series with a diode-array detector and a β-flow detector.[12] The cobalt concentration in the final

solution of purified [4-^{11}C]GABA was determined by a Perkin-Elmer 2380 atomic absorption spectrophotometer equipped with a Cr, Co, Cu, Mn, Fe, Ni, lamp working at 241 nm. Tetrahydrofuran (THF) was distilled from benzophenone and sodium in an atmosphere of nitrogen to a dry glass vessel containing 4-Å molecular sieves. Ethyl acrylate was distilled and stored refrigerated.

γ-amino[4-^{11}C]butyric acid (GABA)

In a septum equipped vial was placed 5-8 mg of kryptofix 2.2.2 (13-21 μmol), 5 μl 0.25 M aqueous potassium hydroxide solution (1.3 μmol) and 400 μl of THF. The reaction mixture was cooled to -30°C and hydrogen [^{11}C]cyanide prepared as briefly described above was trapped on-line during its synthesis. When the trapping was finished, 50-60 μl of ethyl acrylate was added (0.46-0.65 mmol) and the reaction mixture heated at 80°C for 5 min. The septum was then removed and 4-7 mg of cobaltous chloride (30-50 μmol) in 150 μl methanol was added followed by 12-15 mg of sodium borohydride (32-39 mmol) in 200 μl of methanol. Cobalt boride (Co_2B) was obtained immediately as a black precipitate. The selective reduction of the nitrile was carried out at 60°C for 5 min. After the addition of 1 ml of 5 M sodium hydroxide and heating at 145°C with moderate agitation during 6 min, [4-^{11}C]GABA was obtained. The reaction mixture was diluted with water and passed through a C-18 Sep-Pak cartridge (conditioned by washing with 5 ml of ethanol and then 20 ml of water) and eluted with 10 ml of water. The eluate was acidified with 0.8 ml of 12 M hydrochloric acid and passed through a cation-exchange resin (Bio-Rad Ag 50W-X4, 200-400 mesh, 50x5 mm). The column was washed with water (around 20 ml) until insignificant amounts of radioactivity were obtained in the eluate. [4-^{11}C]GABA was then eluted with 0.5 M aqueous ammonia. Following evaporation and addition of saline, pH adjustment and sterile filtration the solution was ready for biomedical application.

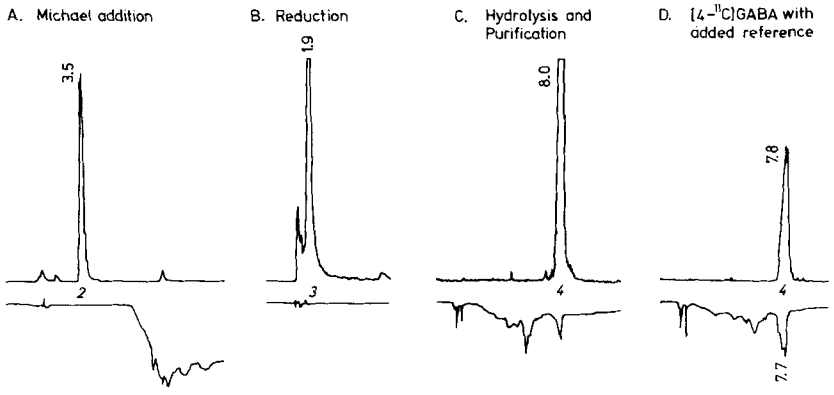
Determination of radiochemical purity and identity

The identity and radiochemical purity of the [4-^{11}C]GABA obtained was determined by liquid chromatography analysis using column A and B. The following conditions were used in the analysis employing column B (LC-NH_2): 0.01 M potassium dihydrogenphosphate/(aceto-nitrile/water, 500/70), 5/95 to 55/45 during 12 min, then isocratic for 3 min, flow 2 ml/min, wavelength 230 nm, column temperature 40°C, retention time for GABA was 7.8 min.

GABA was converted to 1-GABA-2,4-dinitrophenyl-5-L-alanine amide by reacting a small aliquot (approximately 5-10% of the total amount) of the labelled product in 200 μl of 0.2 M

sodium hydrogencarbonate solution with 200 µl of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (1% solution in acetone).[13] The reaction mixture was heated at 60°C for 15 min, quenched by the addition of 50 µl of 2 M hydrochloric acid and analysed by liquid chromatography using column A (C-18) employing the following conditions: 0.05 M ammonium formate pH 3.5/methanol, 80/20 (v,v) linear gradient 0-10 min to 50/50 (v,v) then isocratic for 10 min, flow 2ml/min, wavelength 340 nm, column temperature 40°C. Retention time for the GABA-derivative was 13.1 min. In both systems the signal from the radiodetector was simultaneous (corrected for time delay between the detectors) with the uv-signal from added reference substances. In Fig. 1 four typical chromatograms from the synthesis of [4-^{11}C]GABA are shown.

Fig. 1



Upper row radiodetector signal, lower row uv signal. Chromatogram A and B: C-18 column eluted with 0.05 M ammonium formate pH 3.5 and methanol, linear gradient 80/20 to 50/50 0-10 min, flow 2 ml/min, 40°C, wavelength 254 nm. Chromatogram C and D: LC-NH_2 column eluted with 0.01 M potassium dihydrogenphosphate pH 4.6 and acetonitrile/water (50/7), linear gradient 5/95 to 50/50 0-12 min, flow 2 ml/min, 40°C, wavelength 230 nm.

Determination of the cobalt content

Two standard solutions of cobaltous chloride were prepared; 2 µg/ml and 0.2 µg/ml. These samples together with an aliquot of the [4-^{11}C]GABA preparation were analysed by atomic absorption spectroscopy. The total amount of cobalt in the solution containing the labelled GABA was found to be lower than 0.4 µg.

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