SYNTHESIS OF ^{11}C-LABELLED CHOLINE

Mirko Diksic*, Y. Lucas Yamamoto and William Feindel
Medical Cyclotron Unit, Montreal Neurological Insitute, and
Department of Neurology and Neurosurgery, McGill University,
3801 University Street, Montreal, Quebec, H3A 2B4

SUMMARY

We report the synthesis of ^{11}C-labelled choline using the reaction of "no-carrier-added" ^{11}C-labelled methyliodide and 2-amino-(N,N-dimethyl)-ethanol. After determining the optimum temperature and duration for the reaction, we achieved a radiochemical yield of about 30% and the radioactivity levels needed for PET studies in humans.

Key words: ^{11}C-labelled choline, quaternary ammonium compound, labelled cholinergic transmitter.

INTRODUCTION

A recent review of cholinergic dysfunction in age-related memory disturbances (e.g. Alzheimer's disease and senile dementia of the Alzheimer type) emphasized the importance of the cholinergic system[1]. Reports on the significant reduction in aging brain of acetyltransferase (the enzyme that converts choline into acetylcholine (ACh)), especially in the cerebral cortex and hippocampal formations, have clarified the role of ACh in brain metabolism[2,3].

Choline as a precursor in the synthesis of ACh in the brain is well established[4-6], and it is known that plasma choline is not its only source[7,8]. Study of ACh kinetics in the brain has in fact revealed two kinetically distinct systems for choline transport into neuronal tissue[8,9].

It is difficult to measure with positron emission tomography (PET) the kinetics of ACh in the human brain because its turnover is several times greater than that of other neurotransmitters and because it is distributed into many compartments. We therefore chose to label its precursor, choline, using the reaction

* To whom correspondence should be addressed.

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of no-carrier-added ^{11}C-labelled methyl iodide and 2-amino-(N,N-dimethyl)-ethanol. The synthesis, based on that of quaternary ammonium compounds, reacts an appropriate amine with CH_3I in the presence of a base[10].

Although scans of monkey brain taken after injection of ^{11}C-choline were recently reported[11], no synthesis details were given.

MATERIALS AND METHODS

Chemicals used in our work were of research purity with the exception of the solvents used for high performance liquid chromatography (HPLC), all of which were distilled in glass. (All chemicals and solvents were purchased from regular suppliers.)

^{11}C-methyl iodide was produced by an adaptation of a standard synthesis[12,13]. The precursor for ^{11}C-methyl iodide was $^{11}C-CO_2$, produced by our medical cyclotron via a $^{14}N(p,\alpha)^{11}C$ reaction on nitrogen of research purity. $^{11}CH_3I$ released from the synthesis vessel[12,13] was carried into a reaction flask where it was collected and reacted with 2-amino-(N,N-dimethyl)-ethanol in a solvent (isopropanol, acetone acetonytryl, or methanol) in the presence of a base (K_2CO_3, $KHCO_3$, and in one experiment Et_3N). After the desired amount of $^{11}CH_3I$ was collected in the vessel containing 2-amino-(N,N-dimethyl) ethanol in an appropriate solvent, the reaction vessel was closed and heated in a sand bath ($\sim 70^{\circ}C$) for 10-20 min. Alternatively, the reaction was carried out by stirring the reaction mixture at room temperature for 10-20 min. (The reaction scheme is outlined in Fig 1.) Finally, the solvent was evaporated and the residue dissolved in methanol. The final purification was accomplished by HPLC on an Amino-Spheri-5 column^{+} with CH_3OH-ether (7:3) as an elution solvent (Fig. 2). Thin layer radiochromatography of the ^{11}C-labelled choline was done on Al_2O_3 plates* with a mixture of CH_3COOH, $(CH_3)_2CO$, CH_3OH-, and C_6H_6 (1:1:4:10) as a developing solvent. An alternative purification involved dissolving ^{11}C-ACh in water and filtering it through a reverse phase Sep-Pak column^{++}. The end product of this purification was assessed by HPLC and thin layer radiochromatography.

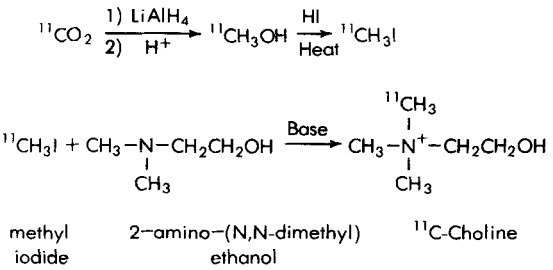

Figure 1: Reaction scheme used in the synthesis of ^{11}C-labelled choline

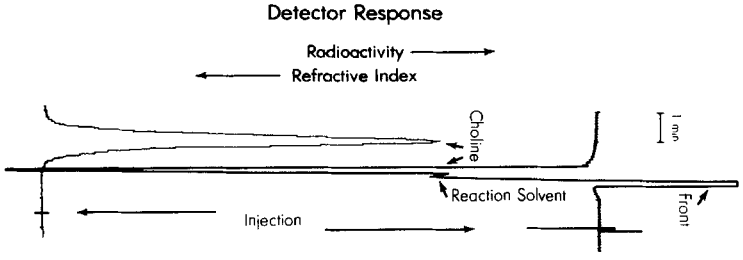

Figure 2: HPLC chromatogram showing radioactive and differential refractive index traces. The elution solvent was a CH_3OH-ether (7:3) mixture flowing at a rate of 1 ml/min. HPLC was done on an Amino Spheri-5 column$^+$.

The specific activity of the final radiopharmaceutical was determined by measuring the absolute amount of radioactivity in an isotope calibrator and dividing the activity by the absolute concentration of choline. The absolute concentration of choline was established by comparing the area of the HPLC elution peak with that of a standard containing 1 μmol/ml of choline. The trace was obtained by using a differential refractive index detector. An HPLC chromatogram showing radioactive and differential refractive index traces is shown in Fig 2. (The starting material and ^{11}C-CH_3I were not retained in the HPLC system.) Since there was not enough choline produced in our synthesis to give a differential refractometer trace, the figure shows a chromatogram obtained after 1 μg/ml of "cold" choline was added. (The procedure for the specific activity determination was similar to the one described in detail for BCNU[14].) The solvent used for the HPLC measurement was the same as that mentioned above in the purification step.

RESULTS AND DISCUSSION

The general reaction scheme used in the synthesis is shown in Fig 1. Although relatively simple, it requires special precautions to exclude moisture. The first step is preparation of ^{11}C-methyliodide, which requires 10-15 min and generally produces a very high chemical yield (about 80%). An acid (HCl or HI) was used to release ^{11}C-CH_3I. When HI is used, ^{11}C-CH_3I can be synthesized in the same vessel. Carbon-11 methyliodide was then reacted with 2-amino-(N,N-dimethyl) ethanol in three different solvents and in the presence of three different bases.

As shown in Table 1, the best yield was achieved when the reaction was done at room temperature using acetone as a solvent and $KHCO_3$ or K_2CO_3 as a base. (Performing reactions at room temperature also obviates the need to handle

manually large amounts of radioactivity.) One reaction was extended for 30 minutes, but the resultant increase in the chemical yield coincided with a reduction in the radiochemical yield (radionuclide decayed by a factor of 1.41). The base $KHCO_3$ gave a marginally better chemical yield. The specific activity of ^{11}C-choline was least when CH_3OH was used as a solvent because of the exchange between the solvent (CH_3OH) and ^{11}C-labelled CH_3I. Where other solvents were used, a specific activity of about 1000 Ci/mmol was obtained. However, it should be pointed out that a high specific activity of choline is not needed because of the large amount of choline present in the blood (12-19 μmol/ml)[15]. This concentration is several orders of magnitude greater than the amount given in the radiopharmaceutical prepared by the synthesis described here.

Since water-solubility of the starting materials is low, the filtration of the final product through a reverse phase Sep-Pak column gives the product a high chemical purity. When the radiochemical yields are converted into chemical yields, it is evident that the reaction described also has a high chemical yield (about 60%).

The synthesis reported here produced ^{11}C-choline at levels needed for PET studies in humans (about 15 mCi after a 30-min synthesis). Radiochemical purity as well as chemical purity of the final radiopharmaceutical exceeded 98%. No starting material was detected in the final radiopharmaceutical.

TABLE 1

Influence of reactive conditions on the yield of ^{11}C-choline

Temperature	Base	Solvent	Radiochemical yield* (%)	Reaction time (min)
room	K_2CO_3	acetone	32	20
room	K_2CO_3	methanol	20	20
room	$KHCO_3$	methanol	22	20
room	$KHCO_3$	acetate	21	15
room	$KHCO_3$	acetone	33	20
room	$KHCO_3$	acetone	25	30
room	$KHCO_3$	acetonitrile	22	20
room	Et_3N	acetone	7	20
70°C bath**	K_2CO_3	acetone	15	20
70°C bath**	K_2CO_3	methanol	10	10
70°C bath**	K_2CO_3	methanol	10	20

* Yield is expressed relative to the $^{11}CH_3I$ produced at the end of a 20-minute synthesis (time from the end of $^{11}CH_3I$ collection), not corrected for radioactive decay.

** In a closed reaction vessel under pressure created by the evaporation of the solvent.

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\+ Brownlee Laboratories, MPLC-7336.

* Aluminium Oxide IB-F sheets, J.T. Baker Chemical Co.

++ Sep-Pak column, C18, Waters Associates.

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