SYNTHESIS OF NO-CARRIER ADDED ^{11}C-LABELLED [METHYL]CHOLINE ANALOGS

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We describe here the synthesis of two[^{11}C-methyl]labelled analogs of choline and the influence of the solvent, addition of a base, and reaction time on the radiochemical yield. The reactions produced equally good yields in methanol and acetonitrile and reached maximum radiochemical yield at about 5-10 min. The addition of a base in the case of [^{11}C-methyl]pyrrolidinocholine increases the radiochemical yield. Optimum radiochemical yields were 30% for[^{11}C-methyl]piperidinocholine and 40% for[^{11}C-methyl]pyrrolidinocholine for two choline analogs.

KEY WORDS: ^{11}C-labelling, [^{11}C]pyrrolidinocholine, [^{11}C]piperidinocholine, ^{11}C-labelled choline analogs.

INTRODUCTION

An alteration in the synthesis of acetylcholine has been postulated to affect the development and progression of Alzheimer's disease (1,2). Two distinct choline-uptake mechanisms (high and low affinity uptake) have been described. We surmised that the high affinity choline uptake system in human brain might be scanned using ^{11}C-labelled choline and positron emission tomography (PET). Friedland et al (3) attempted to scan this high affinity uptake system in monkey and Gauthier et al (4) in humans using[^{11}C-methyl]choline. Unfortunately the results were unsatisfactory because of large amounts of endogenous choline used in the synthesis of acetylcholine. This prompted us to investigate the choline analogs, pyrrolidinochline and piperidinocholine, because they are taken up by the same system as acetylcholine and are substrates for acetylcholine esterase (5).

We have described the synthesis of no-carrier-added[^{11}C-methyl]choline elsewhere (6), and other researchers recently investigated the effect of a possible impurity, 2-(N,N-dimethyl) ethanol, on the uptake of choline by the rat brain (7). Here we describe the synthesis of no-carrier-added ^{11}C-labelled (methyl) analogs of choline, piperidynocholine and pyrrolidinocholine. The synthesis is based on the same principle as that described earlier by us (6) and reported recently (7) for[^{11}C-methyl]choline.

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MATERIALS AND METHODS

1-(2-hydroxyethyl) pyrrolidine (1) and 1-(2-hydroxyethyl) piperidine (3) were purchased from Aldrich Chemical Company and used without further purification. Thin layer chromatography (TLC) and radiochromatography (TLRC) were done on Al_2O_3 plates^{+} using acetic acid-acetone-methanol-benzene (1:1:4:10) as the developing solvent. High performance liquid chromatography (HPLC) was carried out on a reverse-phase (RP-18)$^{++}$ or C-18^{+++} column with water as the elution solvent using a UV-detector at 200 nm and a radioactivity detector with a 50 µl active volume. ^{1}H-NMR spectra were done at 200 MHz on a Varian XL-200 spectrometer using methanol-d_4 as solvent. IR spectra were taken both in KBr pallets or in a nujol mixture using a Perkin-Elmer 297 spectrometer. The mass spectra were obtained with a HP 5980A spectrometer using electron impact ionization.

Preparation of [^{11}C]CH_3I: ^{11}C-labelled methyl iodide was prepared by adapting the method previously described (8,9). ^{11}C-labelled carbon dioxide (no-carrier-added) was produced by irradiating 99.99% nitrogen gas with 10 MeV protons by a ^{14}N (p, α) ^{11}C reaction in a target chamber of about 150 ml at a pressure of 4 Atm (beam off). After irradiation $^{11}CO_2$ was introduced into a vessel containing $LiAlH_4$ in THF cooled in a dry-ice acetone bath. After transferring $^{11}CO_2$ radioactivity to a reduction vessel, the solvent was evaporated to dryness and 0.5 ml of water was added to release methanol which was carried with a stream of helium through hot HI. In the preparative runs the hot HI was added directly to the reduction vessel where $^{11}CH_3OH$ was synthesized. Released $^{11}CH_3I$ was passed through a NaOH (solid) trap and carried by a stream of helium into the reaction vessel.

Synthesis of [^{11}C-methyl] pyrrolidinocholine (2) (Figure 1): $^{11}CH_3I$ prepared as described above was introduced into a reaction vessel containing 0.1-0.4 ml of 1-(2-hydroxyethyl) pyrrolidine (1) dissolved in 1 ml of CH_3CN or CH_3OH. In some experiments a base was added (Table 1). The reaction was continued by stirring and analyzed by TLRC at various times after the introduction of $^{11}CH_3I$. ^{11}C-methyl pyrrolidinocholine was purified by HPLC on a reverse phase (C-18^{+++}) using water as the elution solvent. This was very effective because [^{11}C-methyl]pyrrolidinocholine (2) eluted almost with the front where the starting material had an elution volume of 9 ml. An alternative method of purification was also investigated. After the reaction was completed, the solvent and most of the starting material were evaporated under reduced pressure. The residue was dissolved in an

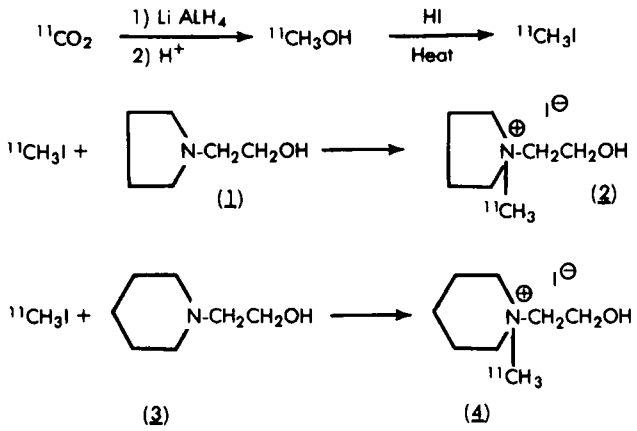

FIGURE 1

Reaction schematics used in the synthesis of ^{11}C-methyl choline analogs

aqueous base (pH = 12; 2 ml) and any excess of starting material was extracted with methylene chloride (2 x 5 ml). After we found the optimum reaction condition for the synthesis of ^{11}C-methyl -labelled pyrrolidinocholine, we evaluated the production runs. In the "preparative" runs we were able to produce 15-40 mCi of [^{11}C-methyl]pyrrolidinocholine.

Thin layer radiochromatography was done on Al_2O_3 plates using as developing solvent acetic acid, acetone, methanol, and benzene in a ratio of 1:1:4:10. The R_f value of the compound 2 was 0.7, identical to the value of an authentic sample**.

TABLE 1

Influence of reaction conditions on the yield of ^{11}C-methyl pyrrolidine
(All reactions done at room temperature)

Base	Solvent	Reaction time	Radiochemical* yield (%)
K_2CO_3	CH_3CN	5 min	40
K_2CO_3	CH_3CN	10 min	35
K_2CO_3	CH_3CN	20 min	25
K_2CO_3	CH_3OH	10 min	10
None	CH_3CN	10 min	15

* Radiochemical yields are expressed at the end of the synthesis relative to the $^{11}CH_3I$ present at t = 0; average of two experiments.

Synthesis of [^{11}C-methyl]piperidinocholine (4) (Figure 1): $^{11}CH_3I$ was introduced into the vessel containing 1 ml CH_3OH or CH_3CN as solvent, 0.2-0.4 ml of 1-(2-hydroxyethyl) piperidine (3), and, in some cases, a base. The reaction mixture was stirred at room temperature for varying periods (see Table 2) and assayed for the yield of the final product. After the end of stirring the solvent was evaporated to dryness and the final product taken up into 1 ml of water.

TABLE 2

Influence of reaction conditions on the yield of ^{11}C piperidinocholine
(All reactions done at room temperature)

Base	Solvent	Reaction time	Radiochemical* yield (%)
None	CH_3OH	5 min	20
None	CH_3OH	10 min	25
None	CH_3OH	20 min	29
None	CH_3CN	20 min	25
K_2CO_3	CH_3CN	20 min	29
K_2CO_3	CH_3OH	20 min	30

* Radiochemical yields at time t (end of synthesis) are expressed relative to the $^{11}CH_3I$ present in the reaction vessel at t = 0; average of two experiments.

Separation of the final ^{11}C-labelled compound from the starting material was done by HPLC on a reverse phase column using water as the elution solvent at a flow rate of 1.5 ml/min. The yields were measured by comparing radioactive amounts of $^{11}CH_3I$ (t = 0) added to the reaction vessel and the final radiopharmaceutical. The radiochemical yields reported in Table 1 and 2 are not corrected for radioactive decay.

The specific activity of the final ^{11}C-labelled material was measured by comparing absolute radioactivity of a sample measured in an isotope calibrator with the absolute concentration of pyrrolidinocholine (2) and piperidinocholine (4), the latter determined by comparing UV detector response at 200 nm to a standard of these two compounds of known concentration.

RESULTS AND DISCUSSION

The reaction scheme used in the synthesis of no-carrier-added [^{11}C-methyl] pyrrolidinochline (2) and piperidinocholine (4) is shown in Fig. 1. The synthesis of ^{11}C-

methyl iodide required about 15 min and produced chemical yields over 90%. In a routine preparation we normally add HI directly to the vessel where $^{11}CH_3OH$ has been prepared, making $^{11}CH_3I$ in the same vessel. Using the experience gained in the synthesis of ^{11}C-labelled choline (6) we investigated reactions only in methanol and acetonitrile. We found that the reaction proceeds swiftly even at room temperature, producing a radiochemical yield after 5 min similar to that at 20 min (see Tables 1 and 2). A prolonged reaction time resulted in an improved chemical yield in the case of piperidinocholine (Table 2); however, the radiochemical yield was not greatly improved because of radioactive decay. As seen from Table 1, the optimal reaction time in the synthesis of [^{11}C-methyl]-labelled pyrrolidinocholine (2) is about 5 min. In a single experiment reactions were done in a closed vial at an elevated temperature (bath of 70° C) but the radiochemical yield did not increase.

In analytical runs the final purification was done by HPLC on a reverse phase column (see Methods). The radioactive fraction had the same elution volume as authentic samples prepared at a millimol level by the same procedure as that described above for the synthesis of ^{11}C-labelled radiopharmaceuticals. In the "cold" synthesis, the products were identified by melting point 228°-232°C for compound (4) (lit. 235°-238°C (10)) and 177°-179°C for compound (2) (lit. 179°-180°C (10)), uncorrected. ^{1}H-NMR of the starting materials (1,3) and products (2,4) revealed the appearance of a singlet corresponding to the N-methyl group (in CD_3OD, δ = 3.2 ppm). It was possible to superimpose the ^{1}H-NMR obtained for the compound (2) we prepared on that of an authentic sample** of pyrrolidinocholine. The IR spectra confirmed the presence of OH (ν = 3395 cm^{-1}) in both choline analogs prepared at a millimolar level.

The HPLC analysis showed that the starting material (3) had an elution volume of 4.2 ml (k' = 2.5), giving very effective separation from the ^{11}C-labelled piperidinocholine (4), which had an elution volume of 1.8 ml (k' = 0.5). There was even better separation between the ^{11}C-labelled pyrrolidinocholine (2) (eluted almost with the front) and the starting material (1) (V_e = 9 ml, k' = 8).

To avoid time-consuming HPLC separation, we developed another way to purify ^{11}C-labelled piperidinocholine (4) and pyrrolidinocholine (2). After the final evaporation of the reaction solvent the residue was dissolved in 2 ml of water (pH = 12) and extracted with 2 x 5 ml of butanol or methylene chloride to remove 1-(2-hydroxyethyl) piperidine (3) and 1-(2-hydroxyethyl) pyrrolidine (1), the starting material. If extraction is used as the mode of

purification, the aqueous layer is neutralized with HCl and 0.5 ml of buffer (pH 7.0) is added. Effectiveness of the extraction method was assessed by HPLC using the same system as above. Less than 1% of the starting material was found in the final radiopharmaceutical when the extraction was used as the final purification step. During the extraction about 8% of the final product was lost. The influence of the base on the yield is not easy to understand because $^{11}CH_3I$ was purified through a solid sodium hydroxide trap to remove any acid. We could not rule out the possibility that the base influenced the yield by deprotinating the amino group, indicating the presence of acid. However, the base generally increased yields more in the synthesis of ^{11}C-labelled pyrrolidinocholine (Table 1) than in the synthesis of piperidinocholine (Table 2).

In preparative runs we were able to produce 15-40 mCi of[^{11}C-methyl]-labelled pyrrolidinocholine (2) and 15-25 mCi of piperidinocholine (4) (ready for the PET study) with specific activity between 1000-2000 Ci/mmol for the preparations done in acetonitrile. The synthesis required about 20 min after the collection of $^{11}C\ CH_3I$. Radiochemical purity, assessed by TLC and HPLC, was over 99%. Chemical purity as assessed by HPLC was also over 99%. We are now evaluating both choline analogs in vivo in monkey and dog.

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\+ Aluminum Oxide B-F, Baker-flex, J.T. Baker Chem. Co., Phillipsburg, N.J.
++ RP-18, Brownlee Labs, Santa Clara, CA
+++ C-18, Microsorb, Rainin Instrument Co., Emeryville, CA
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