Carbon-11 Choline: Synthesis, Purification, and Brain Uptake Inhibition by 2-Dimethylaminoethanol

Mark A. Rosen, Reese M. Jones, Yukio Yano, and Thomas F. Budinger

Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, California

We report an improved method for the synthesis and purification of [^{11}C]methylcholine from the precursors [^{11}C]methyliodide and 2-dimethylaminoethanol (deanol). Preparation time, including purification, is 35 min postbombardment. Forty millicuries of purified injectable [^{11}C]choline were produced with a measured specific activity of >300 Ci/mmol and a radiochemical purity >98%. The decay corrected radiochemical yield for the synthesis and purification was ~50%. Residual precursor deanol, which inhibits brain uptake of choline, is removed by a rapid preparative high performance liquid chromatography (HPLC) method using a reverse phase cyano column with a biologically compatible 100% water eluent. Evaporation alone did not completely remove the deanol precursor. Brain uptake of the [^{11}C]choline product was six times greater after HPLC removal of deanol because doses of less than 1 μg/kg significantly inhibit [^{14}C]choline brain uptake.

J Nucl Med 26:1424–1428, 1985

The central cholinergic system may be involved in several neurological diseases including Alzheimer's disease. Positron emission tomography (PET) could be used to study the brain uptake kinetics and metabolism of carbon-11 (^{11}C) choline in local brain regions of human subjects with these diseases.

The synthesis of the precursor [^{11}C]methyliodide presented here is a modification of procedures described by Comar et al. (*1*). The general synthetic utility of N-methylation using [^{11}C]methyliodide is well established (*2-6*). A synthesis of [^{11}C]choline from [^{11}C]methyliodide and 2-dimethylaminoethanol (deanol) has recently been reported (*7*). The procedure described here incorporates an improved preparative high performance liquid chromatography (HPLC), method using biologically compatible aqueous solvents, for removing unreacted deanol precursor and for purifying the [^{11}C]choline product.

The removal of the deanol precursor is an essential part of the preparation because deanol can compete with choline for brain uptake. The precursor, deanol, in concentrations of 10 to 500 μmol, has been shown by Cornford et al. (*8*) to have a five- to tenfold greater affinity than choline for transport across the blood-brain barrier. Deanol was injected along with [^{14}C]choline in rats to determine the effect of the deanol on the brain uptake of the labeled choline. The preparative HPLC method purified the high specific activity [^{11}C]choline by removing any unreacted deanol precursor and other radioactive impurities. The 5-min brain uptake of the [^{11}C]choline product was measured before and after HPLC purification.

Received Dec. 17, 1984; revision accepted Aug. 22, 1985.

For reprints contact: Mark A. Rosen, MD, Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

MATERIALS AND METHODS

Preparation of [^{11}C]choline

$$^{11}CH_3I + HOCH_2CH_2N(CH_3)_2 \longrightarrow HOCH_2CH_2N(CH_3)_2{}^{11}CH_3{}^+ I^-$$

Carbon-11-methyliodide was prepared following the method of Comar et al. (*1*) with minor modifications. Carbon-11 was obtained from the nuclear reaction $^{14}N(p,\alpha)^{11}C$ by irradiating nitrogen gas* in a 20 cm by 4 cm target (on axis) under 11 atm pressure (160 psi) at a flow rate of 200 cc/min for a period of 30 min with a 25

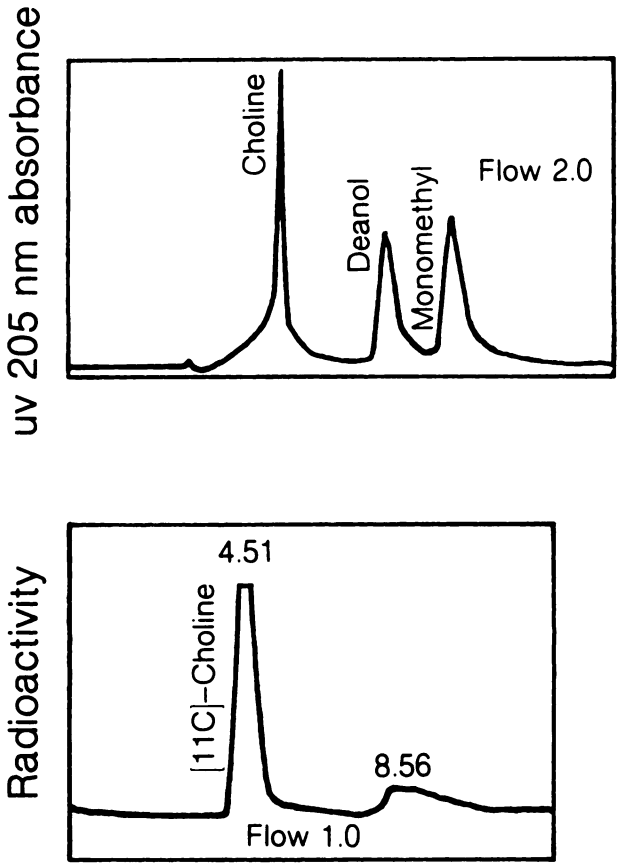

FIGURE 1
Preparative HPLC separation for removing deanol from [^{11}C]choline product employed reverse phase cyano derivatized column [Waters Radial Pack (i)] with 100% water eluent at flow rate of 1.0 ml/min. Shown here is chromatogram of chemical standards (top) and typical preparative separation from which activity peak corresponding to choline was collected

μA beam current of 20 MeV protons‡. The $^{11}CO_2$ was trapped and then bubbled through 0.1*M* $LiAlH_4$ in tetrahydrofuran‡ at −20°C. After evaporating the THF solvent, the conversion from $LiAl(O^{11}CH_3)_4$ to $^{11}CH_3OH$ was done with 0.01*M* H_2SO_4 (instead of pure water) to facilitate the breakup of the particulate aluminum oxide matrix. The $^{11}CH_3I$ was formed by bubbling the $^{11}CH_3OH$ through a second vessel containing refluxing hydroidic acid§. The methyliodide was captured in the final reaction vessel, which contained 500 μg of twice distilled deanol¶ in 4.5 ml anhydrous acetone** cooled to −20°C.

Following the transfer of the activity to the reaction vessel, the isolated vessel was warmed slowly to 70°C over a period of 10 min. The acetone was evaporated in the presence of a vacuum by heating the bath to 100°C. One milliliter of normal saline was added to rinse the [^{11}C]choline through a 0.22 μm Teflon filter‡ into the 2-ml sample loop of the HPLC injector‡‡.

Separation of deanol from choline

A reverse phase cyano column§§ was used with a 100% H_2O eluent at a flow rate of 1.0 ml/min. Ultraviolet (205 nm) and gamma detectors (NaI(Tl) crystals were used to monitor the chemical and radioactivity peaks. The major radioactivity peak coincided with the retention time for the choline (Fig. 1). Retention volumes for methanol, methyliodide, choline, deanol, and 2-mono-methylaminoethanol were 3.2, 3.9, 2.7, 3.7, and 4.5 ml, respectively. The 2-ml fraction collected at the retention volume for choline was passed through a 0.22 μ Teflon filter. The purified hypotonic solution was then combined with an equal volume of buffered hypertonic saline (1.8%) for i.v. injection.

To assay the purity of the final product a second HPLC method used a reverse-phase amino (NH_2) bonded column¶¶ with an eluent of 80% acetronitrile*** and 20% buffer (0.01*M* Na PO_4, pH 4.4) at a flow rate of 1 ml/min. Chemical detection was by uv absorbance at 205 nm††† and gamma detection was made by passing the eluent between a pair of (NaI(Tl)) crystals and observing either coincidence or summed events. Chemical and radioactivity peak areas were measured using a computing integrator‡‡‡. The retention times for methanol, methyliodide, deanol, and choline were 1.6, 1.6, 3.7, 4.3 min, respectively, on the NH_2 HPLC system. This second HPLC method is efficient for the separation of some radioactive impurities such as [^{11}C]methanol and [^{11}C]methyliodide from [^{11}C]choline but not adequate for complete separation of deanol from choline (Fig. 2).

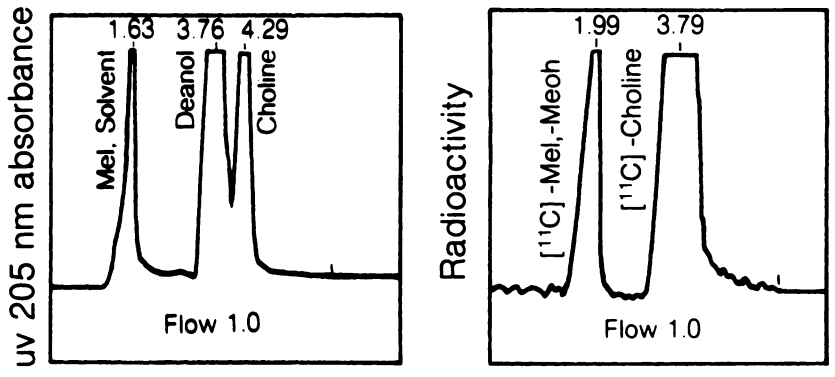

FIGURE 2
Analytic HPLC method, which employed 25-cm amino derivatized(j) column with 80:20 acetonitrile:buffer (0.01*M* Na PO_4, pH 4.3) eluent was used for measuring any [^{11}C]methyliodide, [^{11}C]methanol or other radiochemical impurities that may be present in final product (but not for separating deanol from choline, which was accomplished in the preparative separation). Shown here is chromatogram of chemical standards (left) and of impure [^{11}C]choline product (right) (before preparative HPLC purification)

Paper chromatography was used for further verification of purity using Whatman No. 1 paper in a solvent system of (8:2:1:3) butanol: ethanol: acetic acid: water. The developed paper was scanned for radioactivity or cut into 0.5-cm fractions and counted. Chemical detection was made by staining with 2% phosphomolybdic acid in 1:1 ethanol:chloroform (v/v), followed by a water rinse, and developed with 1% $SnCl_2$ in 3N HCl (*9*)

Animal experiments

Adult male albino rats§§§, 2-3 mo old and 250-300 g body weight were fed water and food¶¶¶ at will. Each rat was anesthetized with the same intraperitoneal dose of sodium pentobarbital (72 mg/kg body weight) and the iliac vein was exposed for injection.

Methyl-labeled [^{14}C]choline****, assumed to be free of deanol, with a specific activity 40–60 mCi/mmol was prepared for injection. On the day of the experiment, the CH_3OH storage solution was evaporated to dryness under a stream of N_2 and reconstituted in normal saline. A series of 5.0 μCi doses of [^{14}C]choline was partitioned into test tubes containing added deanol in various amounts. The rats were injected through the iliac vein with 0.5-ml volumes containing the [^{14}C]choline and added deanol doses from none to 20 μg/kg.

Five minutes after injection the rats were killed and blood was dripped into heparinized vials. The brain was removed, weighed, then homogenized with 4 ml of a solution containing 50% CH_3OH, and 50% of a solution composed of 0.3 N $HClO_4$ and 1 m*M* ethylenediaminetetraacetic acid in H_2O. Duplicate 100 μml fractions of the blood solution and brain homogenate solution were pipetted and counted over 10 min for [^{14}C] activity in copolymer mini vials containing 4 ml of scintillation fluid††††.

At least two animals were measured at each of five deanol doses. Five control rats received [^{14}C]choline without any added deanol. The percent of the injected dose/g of brain and blood was calculated for each rat to compare the effect of the added deanol on the brain uptake of the labeled choline 5 min after the i.v. injection.

The brain uptake of one preparation of [^{11}C]choline was measured in rats before and after HPLC purification (one rat each). One milliliter doses of 155 μCi of unpurified or 17 γrmCi of HPLC purified [^{11}C]choline were injected into different rats as above. Five minutes after injection the rats were killed and percent of injected dose in the brains was determined by gamma counting of the weighed brain tissue.

The approximate deanol and choline concentrations in each of the two [^{11}C]choline preparations using the preparative HPLC separation were measured by comparing the integrated 205 nm (bandwidth 10 nm) absorbance peak area to the peak area of a solution of known concentration. These HPLC measures were also used to determine the approximate specific activity of the [^{11}C]choline. The solution to be measured was concentrated by evaporation under streaming N_2 reconstituted in 100 γrml of HPLC eluent and injected on the HPLC system. The approximate choline concentration was calculated from the integrated uv 205 absorbance peak area.

RESULTS

The addition of methyliodide to deanol to form choline gave a chemical yield of 93%. The overall radiochemical yield for the production of [^{14}C]choline from $^{14}CO_2$ using the procedure described above was 85%. Most of the unincorporated [^{14}C] radioactivity was in the form of [^{14}C]methyliodide.

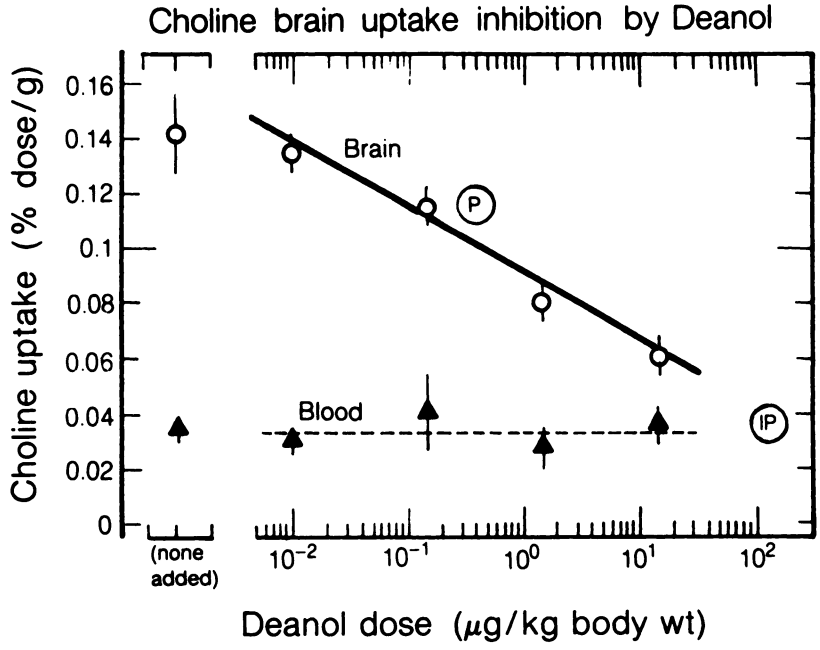

FIGURE 3
Effect of deanol on brain uptake of choline at 5 min after i.v. injection in rats. Labeled [^{14}C]choline was mixed with increasing concentrations of deanol and injected. (O)[^{14}C]choline in brain; (▲)[^{14}C]choline in blood; (IP)Impure [^{11}C]choline brain uptake; (P)HPLC purified [^{11}C]choline brain uptake

For the production of [^{11}C]choline, the overall radiochemical yield was ~60%, after HPLC purification. Before purification, the decay corrected radiochemical yield for the production of [^{11}C]choline from $^{11}CO_2$, was ~75%. The HPLC purified [^{11}C]choline product had a radiochemical purity of >98% as measured by HPLC and paper chromatography. The specific activity was ~300 Ci/mmol choline. Seventy millicuries of prepurified [^{11}C]choline were routinely produced, with 20–40 mCi typically available for use after HPLC purification and preparation for injection.

The brain uptake in the rats of [^{14}C]choline with no deanol was 0.15% of injected dose/g brain 5 min after i.v. injection (n=5). The [^{14}C]choline brain uptake was inhibited 50% with a deanol dose of 2 μg/kg body weight (Fig. 3). The brain uptake of the HPLC purified [^{11}C]choline was 0.12% of injected dose/g brain tissue (n=1) compared with the 0.02% brain uptake for [^{11}C]choline before HPLC purification (n=1).

DISCUSSION

Diksic et al. (*7*) described the synthesis of [^{11}C]choline from deanol by methyliodide addition. They found that acetone is the preferred solvent for the reaction. Their evaporation of the acetone solvent with heat (70 to 100°C) and vacuum removed only 70% of the deanol. The saline solution used to rinse the product from the reaction vessel was found to contain 30% of the original deanol precurser. This amount of deanol if injected along with [^{11}C]choline is sufficient to inhibit by ~ 80% the choline brain uptake in rats.

Our preparative HPLC method separates [^{11}C]choline from any remaining deanol and other radioactive or chemical impurities. Previously reported HPLC methods for separating choline from deanol, use organic solvents for the eluent (*7*). When using such solvents the collected peak fraction must first be evaporated to dryness and then reconstituted in aqueous buffer before i.v. injection. The 100% H_2O eluent and short retention times in the preparative method described here allow for rapid purification along with simple processing for i.v. injection without the need of removing organic solvents.

Care should be taken to ensure that the precursor deanol is free of the monomethyl impurity to obtain good yields of labeled choline. In one [^{11}C]choline synthesis run, radioactivity was found in the form of deanol because the deanol solution used for the synthesis was contaminated with monomethyl-aminoethanol (b.p. 155°C for the monomethyl compared with 135°C for the dimethyl). The preparative HPLC method should remove both unreacted deanol and any [^{11}C]labeled deanol arising from a monomethyl-aminoethanol impurity in the starting product. In one pass through the HPLC, ~ 99% of the deanol is removed.

The brain uptake of the [^{11}C]choline product was measured in rats before and after preparative HPLC purification. The HPLC purified product has a percent brain uptake six times greater than that of the unpurified product (Fig. 3). Cheney and Costa showed that pentobarbital anesthetic reduces choline uptake in brain by affecting the high affinity transport system (*10*). Thus, the absolute brain uptake measured here in rats anesthetized with pentobarbital, may be different than the brain uptake in an unanesthetized animal. The effect of deanol upon brain uptake of choline should occur in both anesthetized and unanesthetized animals, although the anesthesia may affect choline and deanol transport to different extents. Each animal received the same anesthesia in these experiments.

In addition to alteration by anesthesia, the amount of injected dose accumulating in the brain may vary with species and time after injection. A few seconds after i.v. injection we expect about 2% or more of the injected dose to be in the brain (*11*). Previous studies using [^{11}C]choline prepared without purification showed early accumulation in the monkey brain with a subsequent washout from the brain and accumulation in head soft tissues. Thus, the fraction of the injected dose (0.15%) reported here represents only one time point 5 min after the injection. Ten minutes after the injection, 0.08% was reported in the brain of rats (*11*).

CONCLUSION

A synthetic method for the preparation of [^{11}C]methylcholine at high specific activity and a preparative HPLC method using 100% water as the eluent for purification of the product is described. The presence of small amounts of deanol precursor markedly decreases brain uptake of [^{14}C]-labeled choline. Purification of the [^{11}C]choline product with preparative HPLC increased choline brain uptake by a factor of 6 (to ~ 0.15% of injected dose) in the brains of rats 5 min after i.v. injection. This low brain uptake suggests that high doses (e.g., 25 miCi [^{11}C]choline) are needed for adequate statistics in PET imaging studies with humans. The amounts appearing in the brain 1 min after an injection could be 2% or more with only 0.2% remaining after 5 min (*11*). If the moment to moment blood-pool concentration is known, then even with the anticipated low statistics, dynamic PET studies of [^{11}C]choline injected i.v. or intracarotid can be performed to evaluate the high affinity uptake system.

ACKNOWLEDGMENTS

The authors thank Mr. Blake Lasky and Mr. Steven Cramer for their assistance in the animal experiments.

FOOTNOTES

*Matheson Zero Gas (< 0.5 ppm contaminant).

† LBL 88 inch clyclotron.

‡ Aldrich Chemical Co., Anhydrous.

§ Merck reagent grade (twice distilled), Merck & Co., Inc., Rahway, NJ.

¶Eastman/Kodak (twice distilled), Eastman Kodak, Rochester, NY.

**Baker anhydrous analytical grade, Baker Instruments, Bethlehem, PA.

††Millipore Millex SR Teflon Filters, Millipore Corp., Bedford, MA.

‡‡Waters Model U6K HPLC Injector, Waters, Rochester, NY.

§§Waters Radial Pak Cyano (10 × 0.8 cm, 10 micron spherical), Waters, Rochester, NY.

¶¶Waters μm-bondpak "carbohydrate analysis" (amino) 10 μ, 25×0.4 cm, or IBM 5μm amino 25 × 0.4 cm, Waters, Rochester, NY.

***Baker HPLC low uv grade, Baker Instruments, Bethlehem, PA.

†††Waters/schofels uv 450 detector at 0.02 AUFS, Waters, Rochester, NY.

‡‡‡Hewlett-Packard 3390a or IBM CS9000 lab computer running CAPS1.2 chromatography application software, Hewlett-Packard, Andover, MA.

§§§Sprague-Dawley, North Adams, MA.

¶¶¶Purina lab chow, Ralston Purina Company, St. Louis, MO.

****Dupont NEN Medical Products, No. Billerica, MA. (56 mCi/mmol).

††††Amersham PCS liquid scintillation cocktail, Arlington Heights, IL.

REFERENCES

1. Comar D, Cartron JC, Maziere M, et al: Labeling and metabolism of methionine-methyl-^{11}C. *Eur J Appl Radiat Isot* 28:49–52, 1976
2. Christman DR, Finn RD, Karlstrom KI, et al: The production of ultra high activity ^{11}C-labeled hydrogen cyanide, carbon dioxide, carbon monoxide and methane via the ^{14}N(p,alpha)^{11}C reaction (XV). *Int J Appl Radiat Isot* 26:435–442, 1975
3. Marazano C, Maziere M, Breger G, et al: Synthesis of methyl iodide-^{11}C and formaldehyde-^{11}C. *Int J Appl Radiat Isot* 28:49–52, 1977
4. Iwata R, Ido T, Saji H, et al: *Int J Appl Radiat Isot* 30:194–916, 1979
5. Breger G, Maziere R, Knipper C, et al: Automated synthesis of ^{11}C-labeled radiopharmacueticals: Imipramine, clorpromazine, nicotine, and methionine. *Int J Appl Radiat Isot* 30:393–399, 1979
6. David J, Yano Y, Cahoon J, et al: Preparation of ^{11}C-methyl iodide and L-[S-methyl-^{11}C]-methionine by an automated continuous flow process. *Int J Appl Radiat Isot* 33:363–369, 1982
7. Diksic Y, Yamamoto L, Feindel W: Synthesis of ^{11}C-labeled choline. *J Label Compds Radiopharm* XXI:9, 1984
8. Cornford EM, Braun LD, Oldendorf WH: Carrier mediated blood-brain barrier transport of choline and certain choline analogs. *J Neurochem* 30:299–308, 1978
9. Block RJ, Durrum EL, Zweig G: *Paper Chromatography and Paper Electrophoresis,* New York, Academic Press, Inc., pp 352–356, 1958
10. Cheney DL, Costa E: *Biochemical Pharmacology of Cholinergic Neurons.* In *Psychopharmacology,* New York, MA Lipton, et al., eds. Raven Press, 1978
11. Friedland RP, Mathis, CA, Budinger TF, et al: Labeled choline and phosphorycholine: Body distribution and brain autoradiography. *J Nucl Med* 24:812–815, 1983