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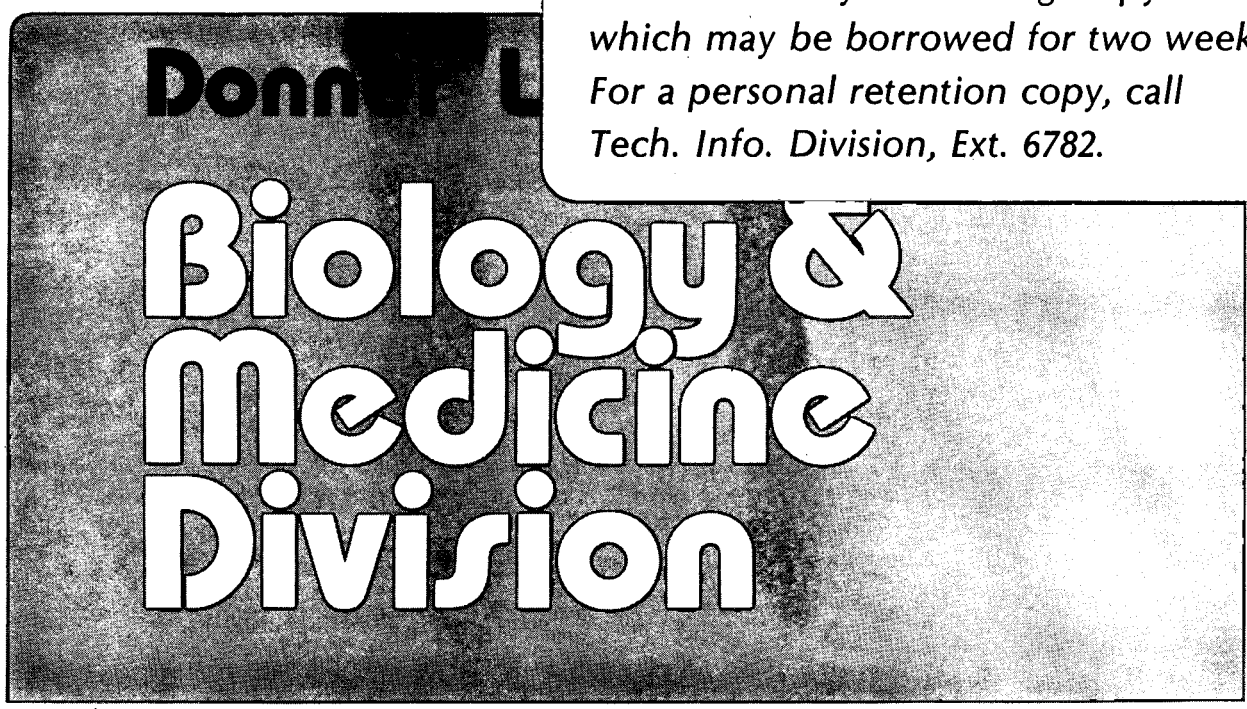

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DISCLAIMER

^{11}C-Choline: Synthesis, Purification and Brain Uptake Inhibition by 2-Dimethylaminoethanol

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Abstract

The central cholinergic system is suspected to be involved in several neurological diseases including Alzheimer's disease. The study of choline metabolism in local brain regions in vivo using positron emission tomography could aid in the investigation of these diseases. We report here the synthesis of [^{11}C-methyl]-choline from the precursors [^{11}C]-methyliodide and 2-dimethyl aminoethanol (deanol). The preparation time is 25 minutes post-bombardment. Seventy mCi of [^{11}C]-choline were produced with a measured specific activity of >300 Ci/mmol and a radiochemical purity greater than 98%. Precursor deanol, which was found to inhibit the brain uptake of choline, is effectively removed by a rapid preparative HPLC method using a reverse phase cyano column with 100% water eluent. Evaporation alone will not completely remove the deanol precursor and amounts of deanol less than 1ug/kg significantly inhibit choline uptake. The brain uptake of the [^{11}C]-choline product was six times greater after HPLC purification. Radiochemical purity was verified by another HPLC method using a reverse phase amino column with an acetonitrile:water (80:20) eluent.

Introduction

The central cholinergic systems have been implicated in a number of diseases, such as tardive dyskinesia, Huntington's chorea, and Alzheimer's disease. Positron emission tomography could be used to study the brain uptake kinetics and metabolism of [^{11}C]-choline in 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[7] and similar to the proceedure published by Diksic et al[7]. The general synthetic utility of N-methylation using [^{11}C]-methyliodide is well established[1-7].

The removal of the deanol precursor is essential as a part of the preparation because it can compete with choline for brain uptake [10]. The precursor, 2-N,N-dimethyl-aminoethanol (deanol), in concentrations of 10 to 500umol, has been shown by Cornford et al.[10] to have a 5- to 10- fold greater affinity for transport across the blood brain barrier than does choline. We find that even trace doses of dimethylaminoethanol can inhibit the brain uptake of choline in rats. A preparative high performance liquid chromatography (HPLC) method was used to purify the high specific activity [^{11}C]-choline and remove any unreacted deanol precursor as well as radioactive impurities.

Materials and Methods

Preparation of [^{11}C]-choline.

$$^{14}N(p,\alpha)^{11}C$$

$$^{11}C + O2 \longrightarrow {}^{11}CO2$$

$$4\ {}^{11}CO_2 + 3\ LiAlH_4 \longrightarrow LiAl(O{}^{11}CH_3)_4 + 2LiAlO_2$$

$$LiAl(O{}^{11}CH_3)_4 + 4\ H_2O \longrightarrow Al(OH)_3 + LiOH + 4\,{}^{11}CH_3OH$$

$$^{11}CH_3OH + HI \longrightarrow {}^{11}CH_3I + H_2O$$

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

[^{11}C] was obtained from the nuclear reaction $^{14}N(p,alpha)^{11}C$ by irradiating nitrogen gas in a 20cm by 4cm 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 25uA beam current of 20MeV protons. The [^{11}C] reacts with trace amounts of O_2 in the target to form ^{11}CO2. High purity nitrogen target gas(a) was filtered through a 4A molecular sieve, and passed through the aluminum target vessel during the irradiation. The gas exiting the target flowed over CuO at 700 C, through a dry ice trap (to capture any water), and finally the $^{11}CO_2$ was trapped by freezing in a coil of copper tubing immersed in liquid nitrogen. After bombardment the liquid nitrogen was removed and the copper tubing trap was allowed to warm slowly to room temperature. The ^{11}CO was carried by N_2 gas, into the first reaction vessel of the synthesis system as shown in Fig.1.

A 3ml solution of 0.1M $LiAlH_4$ in tetrahydrofuran was placed in the first reaction vessel and cooled to -20°C with an ice/ethylene glycol mixture. The $^{11}CO_2$ was delivered from the coil trap to this vessel over a period of approximately 3 min. After complete transfer of the $^{11}CO_2$, the THF sovent was removed by passing a nitrogen gas stream through the solution and heating to 60°C (approximately 4 minutes).

One ml of 0.01M H_2SO_4 in water was added to first vessel, which was then heated to 100°C. The water caused the conversion of the $LiAl(O^{11}CH_3)_4$ to $^{11}CH_3OH$, which was then swept through refluxing HI with the N_2 carrier gas. The H_2SO_4 was added to the water to facilitate the breakup of the particulate aluminum oxide matrix.

$^{11}CH_3I$ was formed when the $^{11}CH_3OH$ was passed through refluxing hydroiodic acid[d] in the second vessel. The methyl iodide vapor was then passed through glass wool and soda lime traps in the third vessel and captured in the final reaction vessel, which contained 500 ug of twice distilled 2-dimethyl-aminoethanol[e] in 4.5 ml anhydrous acetone[f] cooled to -20°C with an ice/ethylene glycol mixture.

Following the transfer of the activity to the reaction vessel, the valves were closed and the isolated vessel warmed slowly to 70°C. The reaction was allowed to proceed for 10 minutes and the acetone evaporated in the presence of a vacuum by heating the bath to 100°C with a resistive heater probe. One ml of normal

saline was added to rinse the [^{11}C]-choline through a 0.22um Millipore filter into a sterile evacuated serum vial or into the 2ml sample loop of the preparative HPLC injector.

Separation of Deanol from Choline: The rinse solution from the final reaction vessel was injected onto the HPLC column in order to separate and remove any unreacted deanol. A reverse phase cyano column(h) was used with a 100% H_2O eluent at a flow rate of 0.5 or 1.0 ml/min. UV (205 nm) [1] and gamma detectors (NaI(Tl) crystals) were used to monitor the chemical and radioactivity peaks. The major radioactivity peak coincided with the UV peak for choline (Fig. 2). At a flow rate of 1.0ml/min the retention times for choline, 2-dimethylaminoethanol (deanol), and 2-monomethylaminoethanol were 2.6, 3.6, and 4.6 min, respectively.

A flow rate of 0.5 ml/min was used for the preparative collection runs and the radioactivity peak corresponding to the retention time for choline was collected. The collected fraction was passed through a 0.22 micron Teflon filter[i] prior to injection. The purified hypotonic solution was then combined with an equal volume of buffered hypertonic saline (1.8%) for injection.

Radiochemical Purity: In order to separate choline from other expected radiochemical impurities such as methyl iodide and methanol, a second HPLC method was used. This method was used to verify the purity of the final product. A reverse phase amino (NH_2) bonded column [j] was used with an eluent of 80%

acetonitrile[k] and 20% water at a flow rate of 1 ml/min. Chemical detection[l] was by UV absorbance at 205nm and gamma detection was made by passing the eluent between 2 sodium iodide crystals and observing either coincidence or summed events. Quantitation was done using a computing integrator[m] or with an IBM CS9000 lab computer equiped with a multi-channel analog input board.[n] The retention times for methyl iodide, 2-dimethylaminoethanol, and choline were 1.6, 3.7, 4.3 min, respectively, on the NH_2 HPLC column. This method is efficient for the separation of radioactive impurities but not adequate for complete separation of deanol from choline (Fig. 3).

For further product purity verification a paper chromatography method was employed [8] using Whatman 1 paper in a solvent system of butanol:ethanol:acetic acid:water (8:2:1:3). The developed paper can be scanned for radioactivity or cut into 0.5cm 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.

Animal Experiments: Adult male albino rats [o], 2-3 months old and 250-300 gm body weight were fed water and food[p] ad libidum. 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 [^{11}C]-choline$^{(g)}$, assumed to be free of deanol, with a specific activity 40-60 mCi/mmol was prepared for injection. 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 the [^{14}C]-choline were partitioned into test tubes containing 2-dimethylaminoethanol in various amounts from none up to doses of 20 ugm/kg. Five hundred μl were drawn into 1 ml syringes for intravenous (i.v.) injection.

Five minutes after injection the rats were decapitated and blood from the neck was collected 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 mM EDTA in H_2O. Duplicate 100 μL 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$^{(r)}$.

At least 2 animals were measured at each of the 5 deanol doses. Five control rats, received [^{11}C]-choline without any added deanol.

The percent of the injected dose/gram 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 ml doses of 155 uCi of unpurified or 17 μCi of HPLC purified [^{11}C]-choline were injected into different rats as above. Five min after injection the rats were sacrificed and the percent of injected dose in the brains was determined by gamma counting of the weighed brain tissue.

The approximate deanol concentrations in each of the two [^{11}C]-choline preparations were measured using the preparative HPLC separation by comparing the integrated 205nm absorbance peak area against the peak area of a solution with known deanol concentration.

Results

The chemical yield for the reaction of methyl iodide with 2-dimethylaminoethanol to form choline was found to be 93% measured chemically. The radiochemical yield of [^{14}C]-choline utilizing $^{14}CO_2$ was determined to be 85%. Most of the unincorporated activity was found in the form of [^{14}C]-methyl iodide.

In the [^{11}C] synthesis approximately 30 percent of the initial $^{11}CO_2$ was incorporated into the final product. The HPLC purified product had a radiochemical purity of >98% as measured by HPLC and paper chromatography. The specific activity was approximately 300 Ci/mmole choline.

In the rats the brain uptake of [^{14}C]-choline with no deanol added was 0.15% of the injected dose/gm brain 5 min after injection. The [^{14}C]-choline brain uptake was inhibited by 50 percent with a deanol dose of 2μg/kg body weight (Fig. 4). The brain uptake of the HPLC purified [^{11}C]-choline was 0.12% of injected dose/gm brain tissue compared with the 0.02% brain uptake for [^{11}C]-choline before HPLC purification. The resulting [^{11}C]-choline brain uptake vs approximate deanol concentration for the two preparations are included with the [^{14}C] results in Fig.4 for comparison.

Discussion

The [^{11}C]-choline synthesis procedure presented here involves the simple addition of [^{11}C]-methyl iodide to a tertinary amine (2-dimethylaminoethanol or deanol) to form the quarternary ammonium salt (ethanaminium,2-hydroxy-N,N,N-trimethyliodide, choline iodide). A method using $^{11}CH_3I$ with ethanol as a solvent followed by ether precipitation has been described for a related reaction [9], but was not used here because the chemical yield was lower with the ethanol solvent. (86% chemical yield compared with 93% for the acetone solvent). In addition, the higher boiling point of ethanol (78.2°C vs. 56.5°C for acetone) is disadvantageous for speed of removal, and the addition of ether to the final reaction vessel is a further inconvenience. Similar findings have been reported by others[7].

Care should be taken to ensure that the precursor 2-dimethylaminoethanol is free of the monomethyl impurity to obtain good yields of labeled choline. In one [^{11}C] synthesis run, activity was found in the form of the precursor 2-dimethylaminoethanol because the deanol solution was contaminated with monomethylaminoethanol (bp 155°C for the monomethyl vs 135°C for the dimethyl). In either case the preparative HPLC method will remove both the unreacted 2-dimethylaminoethanol and any [^{11}C]-labeled 2-dimethylaminoethanol arising from a monomethylaminoethanol impurity in the starting product.

We found that the evaporation of the acetone solvent with heat (70°C) and vacuum removed only 70% of the 2-dimethylaminoethanol. After rinsing the dried product from the vessel with one ml of saline the solution was found to contain 30% of the 2-dimethylaminoethanol precurser, which if not removed is sufficient to inhibit the brain uptake of the labeled choline by 85% as described in Results.

The preparative HPLC method separates the [^{11}C]-choline from any remaining [^{11}C]-methyl iodide and 2-dimethylaminoethanol and other radioactive or chemical impurities that may be present. Previously reported HPLC methods for separating choline from deanol, use biologically incompatible 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 percent H_2O eluent

and short retention times in the preparative method described here allow for rapid purification and further processing for i.v. injection without the need of removing organic solvents.

The brain uptake of the ^{11}C-choline product was measured in rats before and after preparative HPLC purification. The HPLC purified product had a percent brain uptake 6 times greater then that of the unpurified product (Fig. 4). In one pass 99.9 percent of the 2-dimethyl-aminoethanol is removed; a two pass purification may by necessary, however to purify the choline product sufficiently for no brain uptake inhibition from the 2-dimethylaminoethanol contaminant.

Cheney and Costa showed that pentobarbital anesthetic reduces choline uptake in brain by affecting the high affinity transport system (11). 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 the brain uptake of choline is expected to occur in both anesthetized and unanesthetized animals. Each animal received the same anesthesia in these experiments.

In addition to the anesthesia, the amount of the injected dose which accumulates in the brain is expected to 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 (12). Previous studies using [^{11}C]-choline prepared without

purification showed early accumulation in the monkey brain with a subsequent wash-out from the brain and accumulation in head soft tissues. Thus, the fraction of the injected dose of 0.15% we report here represents only one time point 5 minutes after the injection. At 10 min, 0.08% was reported in rats [12].

Conclusion

A synthetic method for the preparation of [^{11}C-methyl]-choline at high specific activity and a preparative HPLC method for purification of the product in injectable form is described. It is shown here that the presence of small amounts of deanol precursor markedly affects brain uptake of the labeled choline. Purification of the [^{11}C]-choline product with the preparative HPLC method improved the choline brain uptake by a factor of 6 to approximatly 0.15% of the injected dose in rats 5 min after injection. This low amount suggests that high doses (eg 25 mCi [^{11}C]-choline) are needed for adequate statistics in PET imaging studies. The amounts which will appear in the brain one minute after an injection could be 2% or more. 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 intra-carotid has promise as a probe of the high affinity uptake system.

Acknowledgments

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Manufacture References:

(a) Matheson Zero Gas (less than 0.5 ppm contaminant)

(b) Aldrich Chemical Co.

(c) Baker HPLC grade made anhydrous with diatomateous earth

(d) Merck reagent grade (twice distilled)

(e) Eastman/Kodak (twice distilled)

(f) Baker anhydrous analytical grade

(h) Waters Radial Pak Cyano (10 x 0.8 cm, 10 micron spherical)

(i) Millipore

(j) Waters u-bondpak "carbohydrate analysis" (amino) 10u, 25x0.4cm, or IBM 5u amino 25 x 0.4cm.

(k) Baker HPLC low UV grade

(l) Waters/Schofels UV450 detector at 0.02 AUFS

(m) Hewlett-Packard 3390a

(n) IBM CS9000 lab computer running CAPS1.12 chromatography application software

(o) Sprague-Dawley

(p) Purina lab chow

(q) New England Nuclear (56 mCi/mmole)

(r) Amersham PCS liquid scintillation cocktail

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Figures:

Fig. 1) System for [^{11}C]-labeling of choline

Fig. 2) The preparative HPLC separation for removing deanol from the [^{11}C]-choline product employed a reverse phase cyano derevatized column (Waters Radial Pack (h)) with a 100 percent water eluent at a flow rate of 1.0 ml/min. Shown here is a chromatogram of chemical standards (above) and a typical preparative separation from which the activity peak corresponding to the choline was collected.

Fig. 3) The analytical HPLC method, which employed a 25cm amino derrvatized column with an acetonitrile:water (80:20) eluent was used for measuring any [^{11}C]-methyl iodide, [^{11}C]-methanol or other radiochemical impurities that may be present in the final product (but not for separating deanol from choline, which was accomplished in the preparative separation). Shown here is a chromatogram of chemical standards and of an impure [^{11}C]-choline product (before preparative HPLC purification).

Fig. 4) The effect of deanol on the brain uptake of choline at 5 minutes after i.v. injection in rats. Labeled [^{14}C]-choline was mixed with increasing concentrations of deanol and injected.

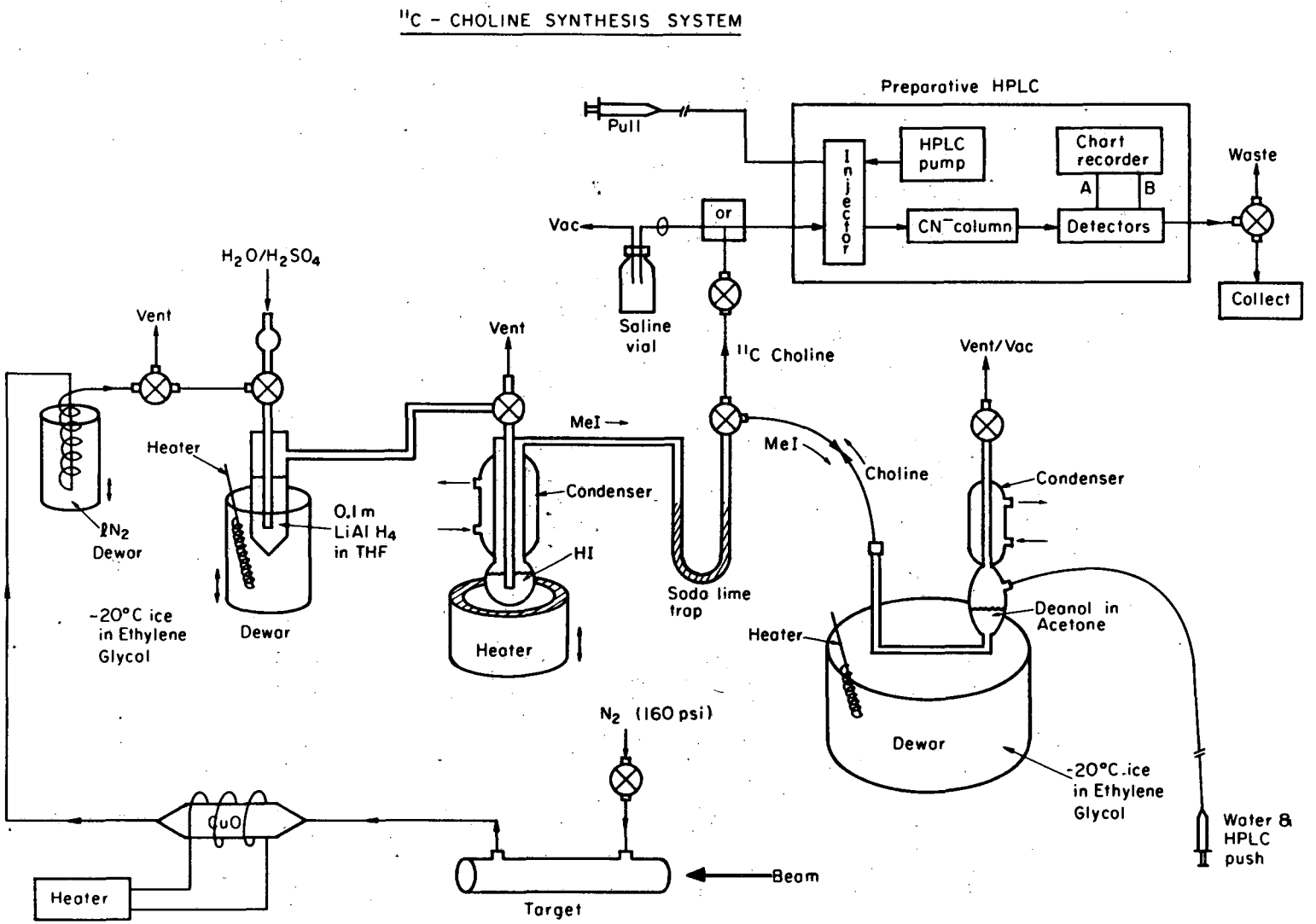
○ :[^{14}C]-choline in brain

▲ :[^{14}C]-choline in blood

Ⓘ :impure [^{11}C]-choline brain uptake

Ⓟ :HPLC purified [^{11}C]-choline brain uptake

FIGURE 1


^{11}C - CHOLINE SYNTHESIS SYSTEM
Preparative HPLC
Pull
HPLC pump
Chart recorder
A
B
Waste
Injector
Vac
or
CN⁻column
Detectors
Collect
H_2O/H_2SO_4
Vent
Saline vial
Vent
^{11}C Choline
Vent/Vac
MeI
MeI
Choline
Heater
Condenser
Condenser
LN_2 Dewar
0.1 m $LiAlH_4$ in THF
HI
Soda lime trap
Deanol in Acetone
-20°C ice in Ethylene Glycol
Dewar
Heater
Heater
N_2 (160 psi)
Dewar
-20°C ice in Ethylene Glycol
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Water & HPLC push
Heater
Target
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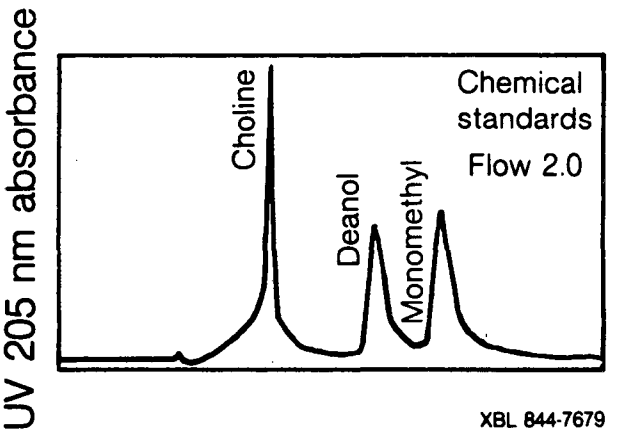
FIGURE 2



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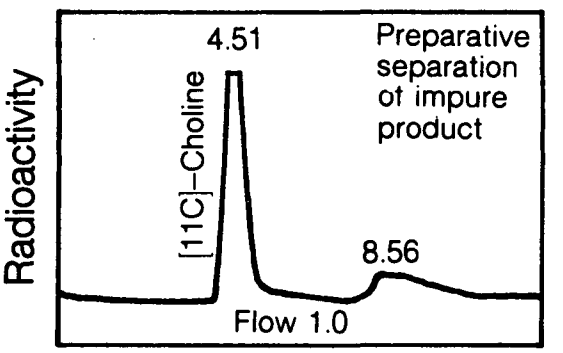

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FIGURE 3

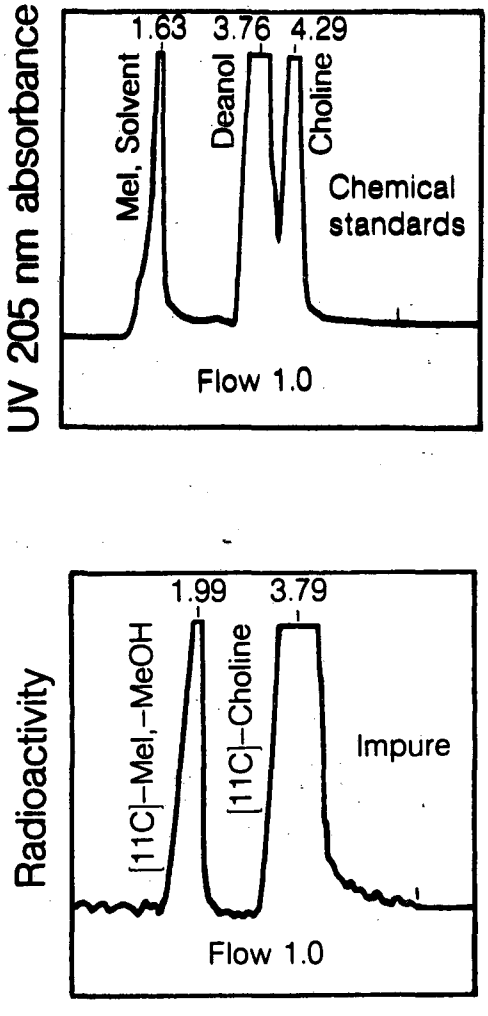

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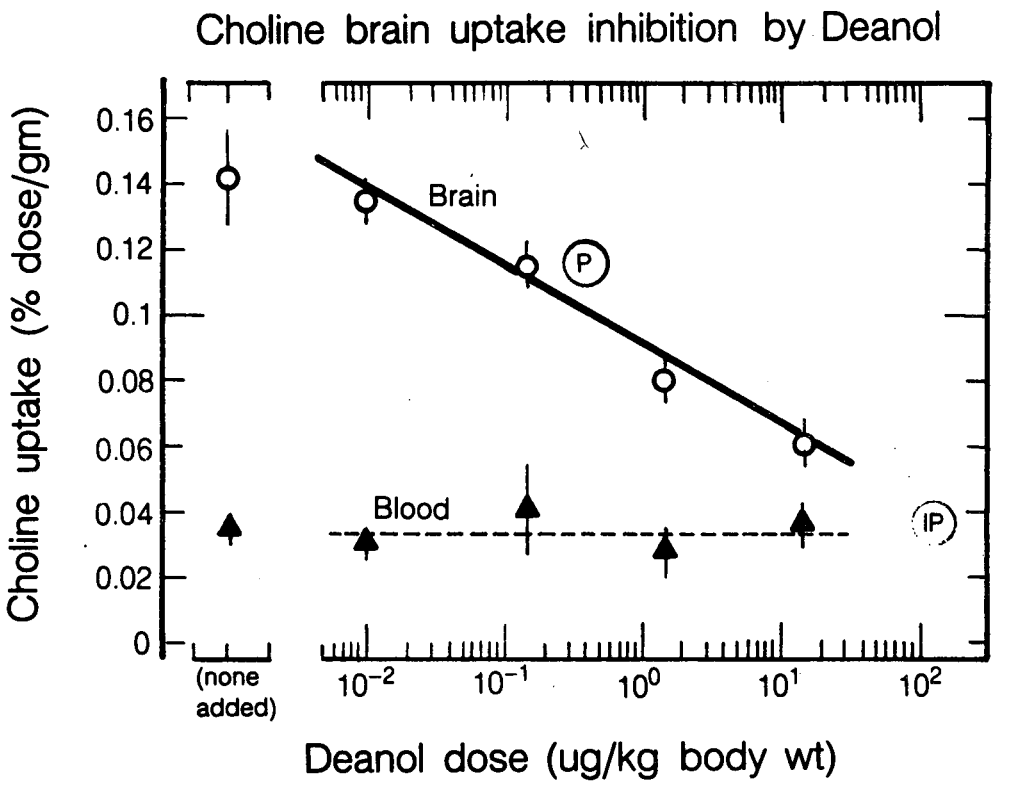
FIGURE 4



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