[N-METHYL-^{11}C]-SCOPOLAMINE: SYNTHESIS AND DISTRIBUTION IN RAT BRAIN*

Manhar M. Vora**, Ronald D. Finn, and Thomas E. Boothe
Mount Sinai Medical Center
Baumritter Institute of Nuclear Medicine
4300 Alton Road, Miami Beach, FL 33140

David R. Liskwosky and Lincoln T. Potter
Department of Pharmacology, University of Miami Medical School
P.O. Box 106189
Miami, Florida 33101

SUMMARY

The muscarinic antagonist [N-methyl-^{11}C]-scopolamine was synthesized by reductive methylation of norscopolamine with H^{11}CHO and sodium cyanoborohydride, and then purified using preparative high performance liquid chromatography (HPLC). Preliminary *in vivo* studies in rat suggest the distribution of this compound to parallel with the muscarine receptor population in the brain.

Key words: [N-Methyl-^{11}C]-Scopolamine, ^{11}C-Formaldehyde, Muscarine Antagonist, Preparative HPLC.

INTRODUCTION

In the central nervous system (CNS), most of the receptors which react with acetylcholine are of the muscarine type. Direct *in vitro* assessment of muscarine receptors in the brain became feasible with the advent of high affinity ligands of high specific radioactivity, including (-)[^{3}H]-3-quinucli-

---

* This work was supported in part by NINCDS grant PO1 NS 15639.

** Author to whom correspondence should be addressed.

0362-4803/83/111229-08$1.00 Received June 20, 1983

dinyl benzilate (1) as well as other ligands (2). Changes in these receptors are known to occur in parkisonism, Huntington's chorea, Alzheimer's disease, tardive dyskinesia and hyperactivity (3-7). Clinical studies may be obtainable using a specific radioligand labeled with a positron-emitting radionuclide, and the positron emission tomograph (8).

This study was undertaken to evaluate the potential utility of some carbon-11 labeled muscarine antagonists to determine the functional status of these receptors. Preliminary studies in rats using ^{3}H-quinuclidinylbenzilate, one of the most potent antimuscarine agents, have indicated that accurate appraisal of receptor distribution in vivo requires a dose sufficient to nearly saturate these receptors (9). Quinuclidinylbenzilate is unsafe at such doses in man, therefore, we have investigated ^{11}C-scopolamine as an alternative label for muscarine receptors(3,10,11).

This paper presents the synthesis of ^{11}C-scopolamine and a preliminary account of its distribution in the rat brain following its intravenous administration.

EXPERIMENTAL

Preparation of H^{11}CHO:

Carbon dioxide [^{11}C] generated from ^{14}N(p, α)^{11}C reaction (12) was reduced to methanol with lithium aluminum hydride, followed by oxidation to formaldehyde over Fe-Mo catalyst. The "no carrier added" H^{11}CHO was distilled into 1 ml of acetonitrile. The radiochemical and purity yield of the H^{11}CHO solution was assessed from the dimedon derivative and the Nash assay (13).

Preparation of ^{11}C-Scopolamine:

The radiopharmaceutical ^{11}C-scopolamine was prepared in three steps: 1) norscopolamine was synthesized from scopolamine via N-demethylation as

described in the literature (14); 2) norscopolamine was subjected to N-methylation with H^{11}CHO in the presence of sodium cyanoborohydride (15); and 3) labeled scopolamine was separated and purified using HPLC and then converted to the injectible radiopharmaceutical. "No carrier added" ^{11}C-formaldehyde in 1 ml of acetonitrile was added to a mixture of 3 mg (10.4 μmoles) of norscopolamine (1), 1 mg(16 μmoles) of sodium cyanoborohydride, and 0.5 μl of glacial acetic acid. The mixture was stirred at room temperature for 10 min. Acetonitrile was removed under reduced pressure and the residue dissolved in 5 ml of diethyl ether. The ether phase was washed with 3 ml of distilled water containing 1 g of anhydrous K_2CO_3; dried briefly over anhydrous Na_2SO_4; and evaporated to dryness. The residue was dissolved in approximately 200 μl of acetonitrile and injected onto a Whatman Partisil PXS 10/25 PAC analytical column (25 cm x 4.6 mm i.d.). The column was eluted with 95% CH_3CN-5% methanol at a flow rate of 2.5 ml/min. The effluent was monitored by a UV detector (240 nm) as well as an on-line NaI detector coupled to a ND-60A multichannel analyzer operated in multichannel scaling mode. Retention time for scopolamine and norscopolamine under these conditions was 5.8 min and 9.0 min, respectively. The fraction corresponding to ^{11}C-scopolamine was collected and evaporated to dryness. The residue was dissolved in 1.5 ml of saline and filtered through a 0.22 μm millipore filter using sterile technique.

Thin layer chromatographic analysis of the product mixture was done on 250 μm silica gel plates (Analtech, Inc., USA); R_f value for scopolamine and norscopolamine was 0.19 and 0.34 in THF: $CHCl_3$ (1:1) and 0.25 and 0.45 in acetone respectively.

Distribution Studies:

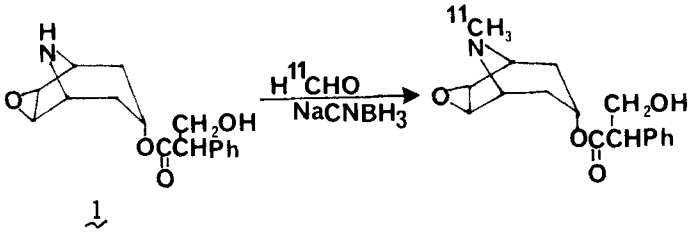
Each of seven rats (175-220g) was given 0.35-0.43 mCi (0.15-0.2mg: 0.5-0.66 μmole) of ^{11}C-scopolamine in 0.5 ml saline via the tail vein. After 20 and 30 minutes the animals were decapitated. Their brains were

rapidly dissected into 6 regions: cortex, basal ganglia, midbrain, cerebellum, pons, and medulla. The tissue was placed in vials to determine the tissue weight, and total radioactivity determined by a well counter. All assays were corrected for radioactive decay.

RESULTS AND DISCUSSION

Synthesis:

Tritium and ^{14}C-scopolamine, like numerous other N-methyl compounds (16), have been prepared from labeled methyl iodide and the corresponding normethyl precursor (reaction conditions (14,17) requiring up to 170 hours at 40°C). The short half-life of carbon-11 mandates a rapid labeling procedure. Attempts to increase the rate of reaction of norscopolamine with ^{11}C-methyl iodide by modifying reaction conditions (e.g., increased temperature and increased polarity of the solvent), resulted in an unsatisfactory yield of the desired product. This may be attributed to the instability of scopolamine and norscopolamine under the reaction conditions employed (18). In contrast, reductive methylation with H^{11}CHO and sodium cyanoborohydride proceeded smoothly and rapidly.



1

This procedure has been employed successfully in preparation of several carbon-11 labeled N-methyl compounds such as nicotine (19) and polyamine analogs (20). In the presence of excess carrier formaldehyde, the yield of scopolamine is almost quantitative within 10 minutes at ambient temperature (Figure 1); whereas, with "no carrier added" formaldehyde, the radiochemical

yield ranged from 30 to 40% based upon the quantity of H^{11}CHO used.

Due to the scale of synthesis, an analytical HPLC column in preparative mode was used to achieve isolation and purification of the radiopharmaceutical. Some peak broadening resulted due to the large volume of injection (∿200 µl on a 250 µl injection loop), but this did not affect the separation.

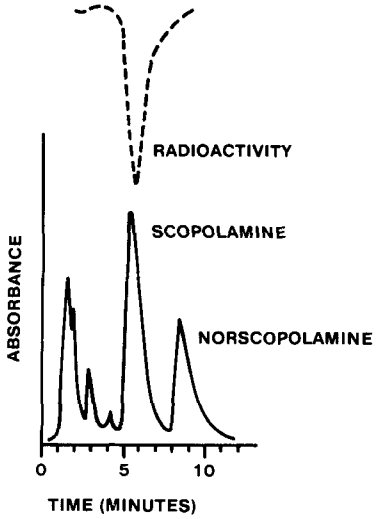


Figure 1. Preparative HPLC Profile (Limiting Quantity of carrier Formaldehyde added)

Unless the product mixture containing labeled scopolamine was washed with aqueous potassium carbonate solution prior to the HPLC separation, the resultant product contained 0.3-0.45 µ moles of cyanide ion, probably as a result of decomposition of the sodium cyanoborohydride. However, the washed product yielded cyanide concentration of approximately 0.06 µ moles.

With this procedure, injectible ^{11}C-scopolamine was prepared within 40 minutes from EOB. The specific activity at the time of injection was 1-4 mCi/ µ mole.

Distribution Studies

Following i.v. injection of the labeled drug, ∿ 0.5% of the injected activity reached the brain 20 minutes postinjection. This is comparable to the

reported value (21) of scopolamine entrance into the brain. It has been reported that the concentration of muscarine receptors in the whole rat brain is in the range of 100 picomoles/g of the brain (7,22-24). Although in the present study, ^{11}C-scopolamine was injected in nanomolar quantities, which is above the saturation level for these receptors, distribution of the activity within the various structures of the brain seems to parallel the muscarine receptor population. (Table 1). At the times examined, the cortex and basal ganglia, which contain the highest concentration of muscarine receptors (21-23) were also the highest in radioactivity; the midbrain, including the hippocampus had an intermediate level of radioactivity; whereas the pons, medulla and cerebellum with the lowest concentration of muscarine receptors had the lowest radioactivity.

TABLE 1

Relative distribution* of ^{11}C-scopolamine within the rat brain

Tissue	Relative distribution per gram of tissue		Muscarine receptor population pmol/g (ref. 23)
	20 min.	30 min.	
Basal Ganglia	100	100	193 (100)**
Cortex	102	80	169 (87.6)
Mid Brain	77	52.5	129 (66.8)
Cerebellum	73.5	43.3	16.8 (8.7)
Pons	71.6	45.4	39.7 (20.6)***
Medulla	75	37	

* Normalized by assigning arbitrary value of 100 to basal ganglia.

** Normalized by assigning arbitrary value of 100 to basal ganglia, for comparison with the experimental values.

*** Combined values for pons and medulla.

The difference in distribution between the latter structures is relatively small especially at 20 minutes postinjection, but does suggest an overall distribution of ^{11}C-scopolamine corresponding to the muscarine receptor population. The blurring of structural differences may reflect the overabundance of unbound drug.

CONCLUSION

^{11}C-Scopolamine can be easily prepared by reductive methylation of norscopolamine with H^{11}CHO and sodium cyanoborohydride. Its distribution in rat brain appears to parallel the muscarine receptor concentration. However, for this compound to be clinically useful the product with high specific activity is needed.

ACKNOWLEDGEMENTS

The authors express their appreciation to Ms. Ana Abreu and Ms. Jan Holle for aiding in manuscript preparation.

REFERENCES

1. Yamamura H. and Snyder S. - Proc. Nat. Acad. Sci. USA 71: 1725 (1974).
2. Birdsall N. and Hulme E. - J. Neurochem. 27: 7 (1976).
3. Heilbronn E. and Bartfai T. - Prog. Neurobiol. 11: 171 (1978).
4. van Woert M. - in Biology of Cholinergic Function, Ed. Goldberg A. and Hanin I., Raven Press, New York, p.567,583 (1976).
5. Weiss B., Foster F. and Kupfer D. - in Biology of Cholinergic Function, Ed. Goldberg A. and Hanin I., Raven Press, New York, p.603 (1976).
6. Silbergeld E. and Goldberg A. - in Biology of Cholinergic Function, Ed. Goldberg A. and Hanin I., Raven Press, New York, p.619 (1976).
7. Bartus R., Dean R., Beer B. and Lippa A. - Science 217: 408(1982).
8. Maziere M., Berger G. and Comar D.-In Applications of Nuclear and

Radiochemistry, Lambrecht R. and Marcos N. ed., Pergamon Press, New York, 1982. p.251.

9. Potter L. and Liskowsky D. - Personal Communication
10. Snyder S., Chang K., Kuhar M. and Yamamura H. - Fedn. Proc. Fedn. Am. Soc. exp. Biol. 34: 1915 (1975).
11. Kloog Y. and Sokolovsky M. - Brain Res. 144: 31 (1978).
12. Christman D.R., Finn R.D., Karlstrom K.I., and Wolf A.P. - Int. J. Appl. Radiat. Isot., 26:435 (1975).
13. Christman D.R., Crawford F.J., Friedkin M., and Wolf A.P. - Proc. Nat. Acad. Sci. USA 69: 988 (1972).
14. Schmidt von H.-L., Werner G. and Krompe G. - Ann. Chemie 688: 228 (1965).
15. Borch R.F. and Hassid A.I. - J. Org. Chem. 37: 1673 (1972).
16. Finn R.D., Boothe T.E., Vora M.M., Hildner J.C., Emran A.M. and Kothari P.J. - Int. J. Appl. Radiat. Isot. - In Print.
17. Werner von G. and Mohammad N. - Ann. Chemie 694: 157 (1966).
18. Werner G. and Schmidt K.-H. - Tetrahedron Lett. 1283 (1967).
19. Berger G., Maziere M., Knipper R., Prenant C., and Comar D. - Int. J. Appl. Radiat. Isotopes: 30: 393 (1979).
20. Welch M.J., Coleman R.E., Straatman M.G., Asberry B.E., Primeau J.L., Fain W.R. and Ter-Pogossian M.M. - J. Nucl. Med. 18:74 (1977).
21. Rehavi M., Yaavetz B., Kloog Y., Maayani S. and Sokolovaky M. - Biochem. Pharmacol. 27: 1117 (1978).
22. Haga T. - in "Pharmacologic and Biochemical Aspects of Neurotransmitter Receptors", Ed. Yoshida H. and Yamamura H., John Wiley, New York, p. 43 (1983).
23. Birdsall N., Hulme E. and Burgen A. - Proc. R. Soc. Lond. B207: 1(1980).
24. Schleifer L. and Eldefrawi M. - Neuropharmacol. 13: 53 (1974).