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Preparation and characterization of (R,S)-[^{76}Br]BrQNB: an analogue of QNB for PET

V. Strijckmans[1*], K.S. Lee[2], C. Loc'h[1], M. Ottaviani[1], B.R. Zeeberg[3], B. Mazière[1].

[1]Service Hospitalier Frédéric Joliot, CEA, DRM, F-91406 Orsay, France

[2]National Institute of Mental Health, Washington DC 20032, USA

[3]George Washington University Medical Center, Washington DC 20037, USA

Summary

(R,S)-[^{76}Br]BrQNB was prepared for imaging mAChR by PET. (R,S)QNB was labelled with bromine-76 by electrophilic substitution of the tributylstannyl precursor using peracetic acid as oxidizing agent. The exchange between bromine-76 and the leaving group occurred in 20 min at room temperature. A chemically and radiochemically pure product was obtained with a final radiolabelling yield of 30%. Preliminary evaluation of pharmacological properties was performed in rats. In brain, biodistribution and autoradiography studies showed that the preferential localization of (R,S)-[^{76}Br]BrQNB was m-AChR rich structures. 6 h p.i. the radioactivity uptake in the posterior cortex was 1% ID/g and the striatum to cerebellum radioactivity ratio was 13.5. Metabolite study revealed that the radiotracer remains unchanged in brain for at least 3 h.

Key Words: QNB, PET, bromine-76, muscarinic cholinergic receptors

* *author for correspondence*

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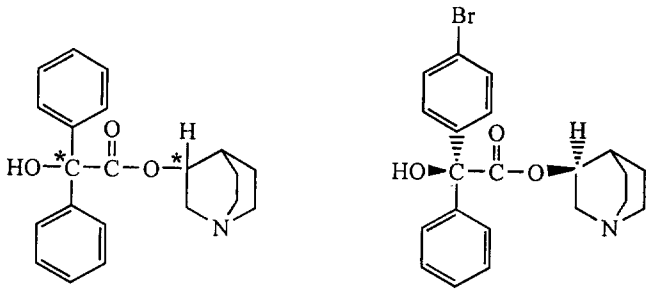
INTRODUCTION

The evaluation of existence and nature of at least four native muscarinic cholinergic receptor subtypes (M_1-M_4) is mainly based on the availability of selective antagonists used in both functional and radioligand binding studies, e.g. pirenzepine (1,2), methoctramine (3,4), AF-DX116 (5), HHSiD (6), 4-DAMP (7), AQ-RA 741 (8) and himbacine (9). This concept of subclassification was recently confirmed by molecular cloning experiments which have revealed the existence of five muscarinic receptor genes (m_1-m_5) (10,11). The m_1, m_2 and m_3 genes correlate pharmacologically to the M_1, M_2 and M_3 receptors, respectively. The M_1 and M_3 receptors have shown to be coupled preferentially to the stimulation of phosphoinositide metabolism while M_2 and M_4 receptors have shown to be coupled preferentially to the inhibition of adenylate cyclase (12). Other effector systems such as voltage-dependent (13) and calcium-dependent potassium channels (14) are also coupled to muscarinic receptor.

The putative role of m-AChR in neurodegenerative disorders such as Alzheimer's disease, Huntington's disease and dementia associated with Parkinson's disease (15-19) has generated considerable interest for the non-invasive mapping of m-AChR. Introduction of non-invasive imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) has made possible the study of neuroreceptors in the living human brain. Such studies have proven useful in the localization and quantification of neuroreceptors and offer insight into the relationship of these receptors in normal and pathological states.

3-Quinuclidinyl benzilate (QNB) (scheme 1) is a high affinity mAChR antagonist that has been labelled with radioiodine (4-^{125}IQNB) (20-22) and ^{11}C (23). Iodine-123-labelled 4-IQNB has been used in SPECT studies in healthy individuals and patients with dementia to image mAChR (24-26). To take advantage of the higher resolution and more accurate quantification of PET, we have prepared (R,S)-[^{76}Br]BrQNB and evaluated its pharmacokinetic properties for imaging mAChR.



Scheme 1

MATERIALS AND METHODS

Radiochemistry

Bromine-76 was produced by irradiation of natural arsenic with a beam of 30 MeV [^{3}He] ions (27). Briefly, after 15 h cooling, the target was dissolved in sulfuric acid followed by oxidation with chromic acid. The radioactive bromine was carried over with a nitrogen stream and trapped in ammonia which was evaporated to dryness and reconstituted in 100 µL water. Radioactivity measurements were made using a Capintec CRC-12 radioisotope calibrator.

Bromine-76-labelled (R)-3-quinuclidinyl (S)-4-bromobenzilate ((R,S)-[^{76}Br]BrQNB) was prepared by electrophilic substitution of the tributylstannyl precursor. [^{76}Br]NH_4 in 100 µl water and 20 µg of tri-*n*-butylstannylQNB precursor in 20 µL of methanol were put into a conical vial and evaporated to dryness. For labelling, peracetic acid (1% in 100 µl acetic acid) and 50 µl hydrogen peroxide were added to the vial and stand in room temperature for 30 min. The labelling process was stopped by evaporating the mixture. The time course of the radiolabelling yield was assessed by radio-TLC using a RP-plate with acetonitrile - 0.1 M ammonium acetate (60:40).

Purification of the radiotracer was carried out by solid phase extraction (SPE) and reverse phase HPLC (RP-HPLC). The residue was taken off by a mixture of water and methanol and

poured into the C18 cartridge (Sep-Pak, Waters). Polar by-products and unreacted [^{76}Br]BrNH$_4$ were washed from the cartridge with 5 ml water. The radiobrominated ligand was further eluted with 3 ml of methanol and isolated by HPLC using a μ-Bondapak C18 column (300 x 3.9 mm, Waters) with a mixture of acetonitrile and 0.1M ammonium acetate (60/40) as the mobile phase at a flow rate of 1 ml/min. The effluent was monitored with an UV detector at 254 nm (M440, Waters) and a Geiger-Muller radioactivity detector. The product was collected and the solvent evaporated. The residue was dissolved in sterile saline. The radiochemical and chemical purity of (R,S)-[^{76}Br]BrQNB was checked by RP-HPLC using similar conditions to those described above.

Determination of specific activity

An aliquot of the final solution of known volume and radioactivity was applied to the analytical reverse-phase HPLC column with the same mobile phase and flow rate condition as described in the radiochemistry. The area of the UV absorbance peak corresponding to the carrier product was measured and compared to a standard curve relating mass to UV absorbance.

Biodistribution

The regional uptake of (R,S)-[^{76}Br]BrQNB was followed for 6 h in Wistar male rats which were injected in the tail vein with 10 μCi (0.37 MBq) of the bromine-76-labelled compound. Rats were sacrificed and brain regions, liver, lung, heart, kidney, muscle, blood were removed and the radioactivity of portions of the tissues was measured in a well γ-counter. The radioactivity concentrations expressed as percent of injected dose per gram of tissue (% ID/g) were plotted versus time.

Autoradiography

The specific *in vivo* brain uptake of (R,S)-[^{76}Br]BrQNB was studied by autoradiography 3 h after injection of 8 MBq of the radiotracer. The brain was recovered, frozen and cut in 20 μm

thickness horizontal and coronal sections with a cryomicrotome (Leitz 1720). The slices were put into X-ray cassettes together with β-radiation sensitive film (Hyperfilm β-max, Amersham) for a 2 day exposure. The films were analyzed using a computerized densitometric system containing a high resolution CCD videocamera and image analysis software.

Metabolite study

The determination of unchanged (R,S)-[^{76}Br]BrQNB in plasma, heart and brain tissues at 60, 120 and 180 min after iv. administration of the radiotracers was performed by TLC analysis. Protein was removed by extraction with acetonitrile. Aliquots of heart and brain tissues (100 mg) and plasma (0.2 ml) were added to acetonitrile (1ml), exposed for 1 min to an ultrasonic probe designed for cell disruption (Vibra-Cells, Sonics & Materials Inc.) and centrifuged. The supernatants were evaporated to dryness and the residues, dissolved in 20 µl of methanol, applied on RP-plates. The TLC-plates were developed with acetonitrile:ammonium acetate 0.1 M in water (60:40) and the radioactivity distribution measured using a static radiochromatogram analyzer (Berthold Co).

RESULTS

Radiochemistry

(R,S)-[^{76}Br]BrQNB was prepared by electrophilic substitution from the tributylstannyl precursor with no-carrier-added [^{76}Br]BrNH$_4$. The exchange between ^{76}Br and the leaving group occurred in 20 min at room temperature (the time-course of the labelling is represented in figure 1). A radiolabelling yield of 50-60% was obtained. Unreacted [^{76}Br]bromide and radiolabelled polar by-products were eliminated using solid phase extraction. 98% of the radiolabelled compound was eluted by methanol. By using an analytical reverse phase column for HPLC, the radiotracer eluted at the retention time of 13 min and before the unlabelled

precursor, avoiding any risk of contamination of the radiopharmaceutical by the tributylstannyl substrate. The total synthesis time including [^{76}Br]bromide preparation was 2.5 to 3 h. The total radiochemical yield was approximately 30-35% and the specific activity using the above reaction conditions was 220 μCi/ nmol (8.1 MBq/nmol). The radiochemical and chemical purity checked by HPLC was > 98%.

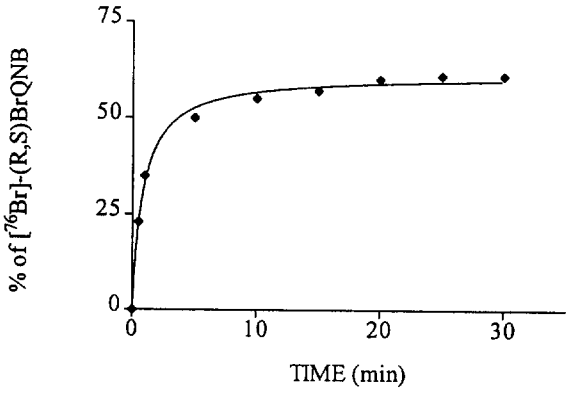


Fig.1

Biodistribution

The time course of the brain distribution of (R,S)-[^{76}Br]BrQNB is represented in figure 2.

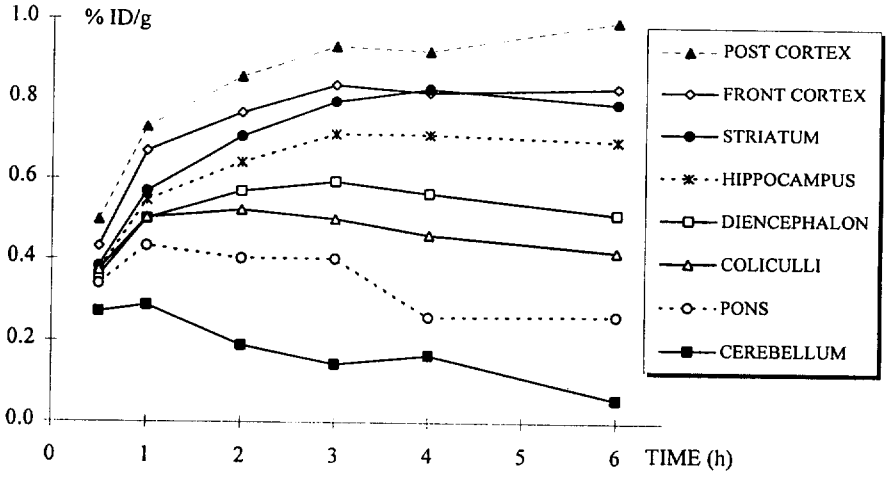


Fig.2

Preferential brain localization of the radiotracer was in the cortex and in the striatum. (R,S)-[^{76}Br]BrQNB accumulates in these regions for at least 6 h. In the hippocampus, maximal radioactivity concentration was reached within 3 h p.i. and is followed by a plateau (0.7 % ID/g) that remain constant for at least 3 h. In the diencephalon and colliculi, the radioactivity peaked at 3 h p.i. and then decreased slowly until the end of the experiment. Due to the low concentration and rapid washout from the cerebellum, the striatum to cerebellum radioactivity concentration ratio was 13.5 at 6 h p.i.

Ex vivo autoradiographic study confirmed this biodistribution study.

In the peripheral organs, the radioactivity concentration remained higher in the lung resulting in a low heart-to-lung radioactivity ratio (0.4) until the end of the study. In blood and plasma the radioactivity concentrations were very low (0.03% ID/g).

Metabolite study

In the cortex, the radiotracer remained unchanged for 3 h. In the heart, (R,S)-[^{76}Br]BrQNB was slowly metabolized since there was still 80% of unchanged radiotracer in this structure 3 h p.i. while it was totally metabolized in the plasma at that time.

Conclusion

We decided to label QNB with bromine-76 because the long half-life ($t_{1/2}$ = 16.2 h) of this positron emitter permits extensive tracer clearance from non specific compartments and prolonged data acquisitions which can be of value in quantification of m-AChR density using pharmacokinetic models. In addition, a carbon-bromine bond (67.5 kcal/mol) is stronger than a carbon-iodine bond (50.5 kcal/mol) which may reduce in vivo dehalogenation.

(R,S)-[^{76}Br]BrQNB was prepared by electrophilic substitution from the tributylstannyl precursor with no-carrier-added [^{76}Br]$BrNH_4$. The radiolabelling and the purification used resulted in radiochemical and chemical pure products. The final radiochemical yield was approximately 30%. Pharmacological results obtained *in vivo* indicate that (R,S)-[^{76}Br]BrQNB is a potential PET radiotracer for studying brain m-AChR receptors.

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REFERENCES

1. Waelbroeck M., Camus J., Tastenoy M. and Christophe J. - FEBS Lett. 226: 287 (1988)
2. Waelbroeck M., Tastenoy M., Camus J., Christophe J., Strohmann C., Linoh H., Zilch H., Tacke R., Mutschler E. and Lambrecht G. - Brain J. Pharmacol. 98: 197 (1989)
3. Melchiorre C., Minarini A., Angeli P., Giardina D., Gulini U. and Quaglia W. - Trends Pharmacol. Sci. 10: 55 (1989)
4. Wess J., Angeli P., Melchiorre C., Moser U., Mutschler E. and Lambrecht G. - Naunyn-Schmiedeberg's Arch.Pharmacol. 338: 246 (1988)
5. Giachetti A., Micheletti R. and Montagna E. - Life Sci. 38: 1663 (1986)
6. Mutschler E. and Lambrecht G. - Trends Pharmacol. Sci. 5: 39 (1984)
7. Barlow R.B., Berry K.J., Glenton P.A.M., Nikolau N.M. and Soh K.S. - Brain J. Pharmacol. 58: 613 (1976)
8. Doods H.N., Willim K., Boddeke H.W.G.M. and Entzeroth M. - Eur. J. Pharmacol. 250: 223 (1993)
9. Lazareno S., Buckley N.J. and Roberts F.F. - Mol. Pharmacol. 38: 805 (1990)
10. Buckley N.J., Bonner T.I. and Brann M.R. - J. Neurosci. 8: 4646 (1988)
11. Peralta E.G., Askenazi A., Winslow J.W., Smith D.H., Ramachandran J. and Capon D.J. - EMBO J. 6: 3923 (1987)
12. Hughes A.R., Martin N.W. and Harden T.K. - Proc. Natl. Acad. Sci. U.S.A 81: 5680 (1984)

13. Adams P.R., Brown D.A. and Constanti A. - J. Physiol. 332: 223 (1982)

14. Yatani A., Hamm H., Codina J., Mazzoni M.R. and Birnbaumer L. - Science 241: 828 (1988)

15. Shimohamata S., Taniguchi T., Fujiwara M. and Kameyama J. - Neurochem. 46: 288 (1986).

16. Mash D.C., Flynn D.D. and Plotter L.T.- Science. 228: 1115 (1985).

17. Lange K.W., Wells F.R., Rosser M.N., Jenner P. and Marsden C.D. - Lancet. 127 (1989).

18. Iversen L.L., Iversen S.D. and Snyder S.H. - Plenum Pubushing Corporation (1988)

19. Holman B.L., Gibson R.E., Hill T.C., Eckelman W.C., Albert M. and Reba R.C. - JAMA 245: 3063 (1985).

20. Gibson R.E., Weckstein D.J., Jagoda E.M., Rzeszotarski W.J., Reba R.C. and Eckelman W.C. - J. Nucl. Med. 25: 214 (1984).

21. Rzeszotarski W.J., Eckelman W.C., Francis B.E., Simms D.A., Gibson R.E., Jagoda E.M., Grissom M.P., Eng R.R., Conklin J.J. and Reba R.C. - J. Med. Chem. 27: 156 (1984)

22. Gibson R.E. - Eds.: Raven Press, New York, NY, 129 (1990)

23. Prenant C., Barre L. and Crouzel C. - J. Labelled Comp. Radiopharm. 27: 1257 (1989)

24. Weinberger D.R., Jones D., Reba R.C., Mann U., Coppola R., Gibson R., Gorey J., Braun S.A. and Chase T.N. - J. Neuropsychiatry Clinical Neurosci. 4: 239 (1992)

25. Owens J., Murray T., McCulloch J. and Wyper D. - J. Labelled Comp. Radiopharm. 31: 45 (1992)

26. Wyper D.J., Brown D., Patterson J., Owens J., Hunter R., Teasdale E. and McCulloch J. - Eur. J. Nucl. Med. 20: 379 (1993)

27. Loc'h C., Mardon K., Valette H., Brutesco K., Merlet P., Syrota A. and Mazière B. Nucl. Med. Biol. 21: 49 (1994)