Positron emission tomographic investigations of central muscarinic cholinergic receptors with three isomers of $[^{76}\text{Br}]\text{BrQNP}$

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Abstract. We studied the potential of three radiobrominated isomers of $\text{BrQNP}$, ($Z(-,-)-[^{76}\text{Br}]\text{BrQNP}$, $E(-,-)-[^{76}\text{Br}]\text{BrQNP}$ and $E(-,+)-[^{76}\text{Br}]\text{BrQNP}$), as suitable radioligands for imaging of central muscarinic cholinergic receptors in the human brain. These radioligands were stereospecifically prepared by electrophilic radiobromodestannylation of the respective tributylstannyl precursors using no-carrier-added $[^{76}\text{Br}]\text{BrNH}_4$ and peracetic acid. Preliminary pharmacological characterizations were determined by biodistribution, autoradiography, competition, displacement and metabolite studies in rats. The $(-,-)$-configuration presented important specific uptakes in brain muscarinic cholinergic receptor (mAChR)-rich structures and in heart, low metabolism rates and an apparent $M_2$ selectivity. The $(-,+)$-configuration revealed more rapid clearance, lower uptake, a higher metabolism rate and an apparent $M_1$ selectivity. Reversibility of the binding was confirmed for the three radiotracers. Positron emission tomography in the living baboon brain revealed high and rapid uptake in the brain and accumulation in the mAChR-rich structures studied. At 30 min p.i., the $E(-,-)$-radiotracer reached a plateau in cortex, pons and thalamus with concentrations of 29%, 24% and 19% ID/l, respectively. $Z(-,-)-[^{76}\text{Br}]\text{BrQNP}$ also accumulated in these structures, reaching a maximal uptake (27% ID/l) in the cortex 2 h p.i. At 5 min p.i. a plateau (17% ID/l) was observed in the cortex for the $E(-,+)-[^{76}\text{Br}]\text{BrQNP}$; by contrast, the other structures showed slow washout. After 3 weeks, the $(-,-)$-radiotracers were studied in the same baboon pretreated with dexetimide (1 mg/kg), a well-known muscarinic antagonist. In all the mAChR structures, the highly reduced uptake observed after this preloading step indicates that these radiotracers specifically bind to muscarinic receptors. $Z(-,-)-[^{76}\text{Br}]\text{BrQNP}$, which is displaced in higher amounts from $M_2$ mAChR-enriched structures, reveals an $M_2$ affinity. The two isomers having the $(-,-)$-configuration are potential probes for investigating central muscarinic receptors. The absolute configuration on the acetate chiral centre influences their muscarinic subtype selectivity and the cis-trans isomerism of the vinyl moiety affects their specific fixation.

Key words: $[^{76}\text{Br}]\text{BrQNP}$ – Muscarinic receptors – Positron emission tomography – Radioligands


Introduction

The classification of the muscarinic cholinergic receptor (mAChR) into subtypes has attracted a great deal of experimental attention for its theoretical and wide-ranging therapeutic and diagnostic implications. Available evidence indicates that cholinergic transmission is implicated in memory and learning [1, 2]. In vitro, in the most prevalent memory disorder, Alzheimer’s disease, marked decreases in the presynaptic $M_2$ mAChR subtype in the cortex and the hippocampus were observed whereas postsynaptic muscarinic receptors were unchanged [3] or even upregulated [4]. A decrease in $M_2$ mAChR density has also been observed in vitro in Huntington’s disease and in dementia associated with Parkinson’s disease.

The introduction of non-invasive imaging techniques such as positron emission tomography (PET) and single-photon emission tomography (SPET) has made possible the study of neuroreceptors in the living human brain. Such studies have proven useful in the localization and quantification of neurotransmitters and offer insight into the relationship of these receptors in normal and pathological states [5]. Muscarinic radiotracers which have been developed for brain PET investigations include $[^{11}\text{C}]\text{quinuclidinyl benzilate (QNB)}$ [6], $[^{11}\text{C}]\text{benztropine}$ [7], $[^{11}\text{C}]\text{dexetimide}$ [8], $[^{18}\text{F}]\text{fluorodextemide}$ [9] and $[^{76}\text{Br}]\text{bromodextemide}$ [10].
The demand for SPET tracers has seen the development of a recently reported analogue of QNB in which a phenyl group has been replaced by a vinyl iodide moiety (IQNP) [11]. Pharmacological studies demonstrated that [125I]IQNP ([125I]-labelled 1-azabicyclo[2.2.2]oct-3-yl-\(\alpha\)-(1-iodo-1-propen-3-yl)-\(\alpha\)-phenylacetate) retained a high in vitro binding affinity for mAChR, passed the blood-brain barrier and displayed high specificity for mAChR in vivo. Evaluation of the stereoisomers of IQNP has shown that the affinity and the mAChR subtype selectivity are influenced by the absolute configuration at the two chiral centres and the vinyl iodide stereochemistry [12-14].

Since bromine behaves in an analogous manner to iodine, similar labelling reactions can be used. In addition, as the carbon–bromine bond (67.5 kcal/mol) is stronger than the carbon–iodine bond (50.5 kcal/mol) in vivo dehalogenation may be reduced. The long half-life (\(t_{1/2}=16.2\) h) of the position emitter bromine-76 permits extensive tracer clearance studies from non-specific compartments and prolonged data acquisitions which can be of value in the quantification of mAChR density using biomathematical models. We have evaluated the influence of the absolute configuration (acetate chiral centre) and the cis-trans isomerism (vinyl moiety) on the pharmacological properties of the 76Br-labelled isomers, and especially selectivity towards the various muscarinic receptor subtypes. To take advantage of the higher resolution and the more accurate quantification of PET, we have also evaluated the pharmacological properties in rats and in baboons of the 76Br-labelled Z(\(-\),\(-\))-\[76Br\]BrQNP, E(\(-\),\(-\))-\[76Br\]BrQNP and E(\(-\),\(+\))\[76Br\]BrQNP isomers of IQNP with the aim of determining their potential use for imaging mAChR in vivo in humans.

**Materials and methods**

*Chemicals.* The preparation of the stannylated precursors for the radiosynthesis has been described previously [13]. Dextetimide hydrochloride and (+)-butacalamol were obtained from Janssen (Belgium), methoctramine, haloperidol and ketanserine from RBI (USA).

*Radiochemicals.* Z(\(-\),\(-\))- and E(\(-\),\(-\))-\[76Br\]BrQNP were synthesized by radioiodination via an electrophilic deamination of the respective azabicyclo[2.2.2]oct-3-yl-\(\alpha\)-hydroxy-\(\alpha\)-phenyl-\(\alpha\)-(1-tributylstannyl-1-propen-3-yl)acetate precursor with no-carrier-added \[76Br\]BrNH\(_2\) using peracetic acid for oxidation [15]. The purification and isolation of the radionucides from the reaction mixture were carried out by reversed phase high-performance liquid chromatography (μBondapak C18, 300x3.9 mm, Millipore Waters) with acetoniitre/0.1 M ammonium acetate buffer (50/50) as the mobile phase. Radiochemical and chemical purities of the radionucides were 98%. Specific activity values were approximately 250 mCi/μmol (9.25 GBq/μmol).

1 The first designation refers to the quinuclidinyl centre and the second to the acetate centre

*Animals.* Procedures were in strict accordance with the recommendations of the EEC (86/909/CEE) and French National Committee (decret 87/848) for the care and use of laboratory animals.

**Rat biodistribution studies.** The tissue distribution of the three 76Br-labelled isomers was evaluated over 6 h in groups of Wistar male rats (n=3) (180 g) which were injected in a lateral tail vein with 0.5 MBq of the radiotracers. The rats were sacrificed at 0.5, 1, 2, 3, 4 or 6 h post injection. Brain, liver, lung, heart, blood, kidney and muscle were removed. The brain structures were dissected and the radioactivity of aliquots was measured. The radioactivity concentrations were expressed as percent of injected dose per gram of wet tissue (% ID/g).

The specificity of the in vivo brain uptake of the radiotracers was investigated in competition experiments by co-injection of the following ligands with the labelled compounds: 1 mg/kg dextetimide (non-subtype selective muscarinic antagonist), 5 mg/kg methoctramine (peripheral M\(_2\) muscarinic antagonist), 5 mg/kg haloperidol (dopaminergic and sigma antagonist), 5 mg/kg (+)-butacalamol (D2/D4, dopamine antagonist) or 5 mg/kg ketanserine (5-HT\(_2\) serotoninergic antagonist). The rats (n=3) were sacrificed 2 h after injection; the brain structures were recovered and their radioactivity concentrations determined.

The reversibility of the in vivo binding of the radioligands was studied in displacement studies by injection of 1 mg/kg of dextetimide and 5 mg/kg of QNB 1 h after the i.v. administration of the 76Br-labelled QNP isomers. The rats (n=3) were sacrificed 0.5 h after the dextetimide or QNB injections. Brain, heart and blood were removed and their radioactivity concentrations calculated as described above.

**Metabolite studies in rats.** The determination of unchanged Z(\(-\),\(-\))-\[76Br\]BrQNP, E(\(-\),\(-\))-\[76Br\]BrQNP and E(\(-\),\(+\))\[76Br\]BrQNP in plasma, heart and brain tissues at 1, 2 and 3 h after i.v. administration of the radiotracers was performed by radio-thin layer chromatography (radio-TLC) analysis. At the same times, the percentage of unchanged E(\(-\),\(+\))\[76Br\]BrQNP in plasma was also determined in rats treated with (+)-butacalamol (5 mg/kg) or haloperidol (5 mg/kg). For protein elimination, aliquots of heart and brain tissues (100 mg) and plasma (0.2 ml) were added to acetoniitre (1 ml), exposed for 1 min to an ultrasonic probe designed for cell disruption (Vibra-Cells, Sonics & Materials Inc.) and centrifuged. Radioactivity of the precipitates was measured to quantify the acetoniitre extraction efficiency. The supernatants were evaporated to dryness and the residues dissolved in 20 μl of methanol and applied on C18 plates (Merck). The TLC plates were then developed with acetoniitre: ammonium acetate 0.1 M in water (60:40) and the radioactivity distribution measured using a static radiochromatogram analyser (Berthold Co).

**Autoradiography.** The specific in vivo brain uptake of the three [76Br]-labelled isomers was studied by autoradiography after injection of 8 MBq of the radiotracers. The rats (n=1 for each isomer) were sacrificed 4 h, 3 h and 1 h after injection of Z(\(-\),\(-\))-\[76Br\]BrQNP, E(\(-\),\(-\))-\[76Br\]BrQNP and E(\(-\),\(+\))\[76Br\]BrQNP, respectively. The brains were removed, frozen and cut in 20-μm thick horizontal sections passing through the frontal cortex, striatum, thalamus, hippocampus and cerebellum 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 analysed using a computerized densitometric system containing a high-resolution CCD videocamera and image analysis software.