



EVALUATION OF Z-(R,R)-IQNP FOR THE POTENTIAL IMAGING OF m2 mAChR RICH REGIONS OF THE BRAIN AND HEART

D. W. McPherson, M. Greenbaum, H. Luo, A. L. Beets, F. F. Knapp, Jr.

Nuclear Medicine Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831-6229

(Received in final form October 1, 1999)

Summary

Alterations in the function or density of the m2 muscarinic (mAChR) subtype have been postulated to play an important role in various dementias such as Alzheimer's disease. The ability to image and quantify the m2 mAChR subtype is of importance for a better understanding of the m2 subtype function in various dementias. Z-(R)-1-Azabicyclo[2.2.2]oct-3-y (R)- α -hydroxy- α -(1-iodo-1-propen-3-yl)- α -phenylacetate (Z-(R,R)-IQNP) has demonstrated significant uptake in cerebral regions that contain a high concentration of m2 mAChR subtype in addition to heart tissue. The present study was undertaken to determine if the uptake of Z-(R,R)-IQNP in these regions is a receptor mediated process and to identify the radiospecies responsible for binding at the receptor site. A blocking study demonstrated cerebral and cardiac levels of activity were significantly reduced by pretreatment (2-3 mg/kg) of (R)-3-quinuclidinyl benzilate, dextetimide and scopolamine, established muscarinic antagonists. A direct comparison of the cerebral and cardiac uptake of [I-125]-Z-(R,R)-IQNP and [I-131]-E-(R,R)-IQNP (high uptake in m1, m4 rich mAChR cerebral regions) demonstrated Z-(R,R)-IQNP localized to a higher degree in cerebral and cardiac regions containing a high concentration of the m2 mAChR subtype as directly compared to E-(R,R)-IQNP. In addition, a study utilizing [I-123]-Z-(R,R)-IQNP, [I-131]-iododextetimide and [I-125]-R-3-quinuclidinyl S-4-iodobenzilate, Z-(R,R)-IQNP demonstrated significantly higher uptake and longer residence time in those regions which contain a high concentration of the m2 receptor subtype. Folch extraction of global brain and heart tissue at various times post injection of [I-125]-Z-(R,R)-IQNP demonstrated that approximately 80% of the activity was extracted in the lipid soluble fraction and identified as the parent ligand by TLC and HPLC analysis. These results demonstrate Z-(R,R)-IQNP has significant uptake, long residence time and high stability in cerebral and cardiac tissues containing high levels of the m2 mAChR subtype. These combined results strongly suggest that Z-(R,R)-IQNP is an attractive ligand for the *in vivo* imaging and evaluation of m2 rich cerebral and cardiac regions by SPECT.

Key Words: Z-(R,R)-IQNP, radiotracer, muscarinic receptor, brain, heart

Corresponding Author: D.W. McPherson, Ph.D., Oak Ridge National Laboratory, Post Office Box 2008, Building 4501, Oak Ridge, TN 37831-6229, TEL (423) 576-6707; FAX (423) 574-6226; E-mail phm@ornl.gov

The muscarinic acetylcholinergic receptor (mAChR) complex has been well characterized, contains five subtypes identified by cloning techniques (m1, m2, m3, m4, m5) (1) and are located in various cerebral structures in differing concentrations (2). Change in the function or density of the various subtypes have been implicated in aging, sudden infant death syndrome (SIDS), learning disabilities, memory, sleep disorders, alcoholism and various dementias such as Alzheimer's (AD) and Parkinson's disease (3-16). Decreased levels of the m2 mAChR subtype, located primarily at presynaptic cholinergic terminals, in the frontal cortex and hippocampus have been suggested to play an important role in the early progression of AD (17,18). Amyloid plaques in patients with Down's syndrome and AD have also been observed in cerebellar and brain stem tissue, regions containing a high concentration of the m2 subtype, although the role or severity of the disease process is not known (19,20). Medial pontine reticular formation primarily involves the m2 mAChR subtype and modulates level of behavior arousal, somatic motor tone and pain sensation (21). It was also observed the cholinceptive pontine neurons play an important role in the control of rapid eye movement sleep (22,23). In addition, the m2 mAChR subtype is the primary subtype in the heart and is implicated to play a role in chronic Chagas' disease and idiopathic dilated cardiomyopathy (24-26). Therefore, the development of ligands for the non-invasive evaluation of m2 subtype mAChR involving cerebral and cardiac mAChR utilizing nuclear medicine techniques is of importance for a better understanding of the role of the mAChR subtypes in various diseases to afford improved diagnosis and cost effective modalities of treatment.

We recently reported a new ligand, azabicyclo[2.2.2]oct-3-yl α -1-hydroxy- α -(1-iodo-1-propen-3-yl)- α -phenylacetate (IQNP, Figure 1), that demonstrates high cerebral and cardiac uptake (27). Upon resolution of the various isomers, E-(R,R)-IQNP demonstrated a selective m1, m4 *in vitro* binding affinity and *in vivo* selective uptake in cerebral regions containing m1, m4 mAChR subtype at 6 hours post injection.

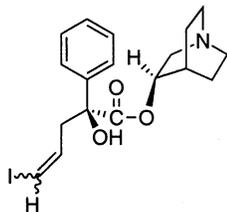


Fig. 1

In contrast, Z-(R,R)-IQNP demonstrated a high non-subtype selective binding affinity *in vitro* and high uptake in all cerebral regions in addition to the heart *in vivo* (28-30). We now report a more detailed *in vivo* biodistribution and *ex vivo* metabolic study of Z-(R,R)-IQNP to evaluate the potential for cerebral and cardiac imaging in various regions containing a high concentration of m2 mAChR subtype by SPECT.

Methods

General Sodium iodine-125 (I-125, specific activity 17.4 Ci/mg) and sodium iodine-131 (I-131, specific activity 7.7 Ci/mg) were purchased from New England Nuclear. Sodium iodine-123 (I-123, specific activity 1900 Ci/mg) was purchased from MDS Nordion. Dexetimide hydrochloride (DEX), naloxone hydrochloride (NAL), (+)- and (-)-butaclamol hydrochloride ((+)- and (-)-BUT) and scopolamine hydrobromide (SCO) were purchased from Research Biochemicals Inc. (R)-3-quinuclidinyl benzilate (QNB) was prepared as reported previously (31). Females Fischer VAF rats (125-150 kg) were purchased from Charles River. Analysis for metabolites by high performance liquid chromatography (HPLC) was performed utilizing a mobile phase of methylene chloride:(ethanol + 1% triethylamine) [98:2], flow rate of 2.5 ml/min, μ Porasil column (3.9 mm X 30 cm) (Waters Inc.), Model 510 HPLC pump (Waters Inc.), Model 454 variable UV detector (254 nm) (Waters Inc.) and a Model 170 radioisotope flow detector (Beckman Instruments Inc.). Radiochemical thin layer chromatographic (TLC) analysis was performed using 250-micron layers of silica gel coated on aluminum sheets

(Merck, Inc.). The plates were marked into ten equal sections and the sample applied. After development and drying, the plates were cut into 10 equal sections and counted in a Packard Minaxi 5000 sodium iodide auto gamma counter.

Radiolabeling E- and Z-(R,R)-IQNP were radiolabeled with either I-123, I-125 or I-131 utilizing E- or Z-tributylstannyl intermediate, iododexetimide (IDEX) was radiolabeled with I-131 utilizing a trimethylsilyl intermediate and R-3-quinuclidinyl S-4-iodobenzilate (4IQNB) was radiolabeled with I-125 utilizing a tributylstannyl intermediate as previously described (28,32,33). The specific activity of [I-123]-Z-(R,R)-IQNP and [I-125]-4IQNB were calculated to be greater than 1000 mCi/ μ mol and approximately 500 mCi/ μ mol for [I-131]-IDEX by comparing to standards the HPLC UV trace (254 nm) of the crude mixture during final HPLC purification.

In Vivo Biodistribution Evaluation The animal care and use procedures were in accordance with the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act and were reviewed and approved by the Oak Ridge National Laboratory Animal Care and Use Committee (ACUC). For blocking studies, [I-125]-Z-(R,R)-IQNP was dissolved in 100 μ L of ethanol, 100 μ L of a 0.1 N HCl solution added and the volume brought up to 10 mL with normal saline. Following filtration through a 0.22 μ Millipore filter, the solution was administered by intravenous injection (0.5 mL) into a lateral tail vein of metophane-anesthetized rats (n=5 rats/group, 4-6 μ Ci) 1 hour post injection (0.2 mL) of a saline solution of SCO (2.7 mg/kg), QNB (2.7 mg/kg) or 30 minutes post injection of a saline solution of NAL (3.0 mg/kg), DEX (3.2 mg/kg), (+)-BUT (3.4 mg/kg) or (-)-BUT (3.4 mg/kg). For a control, a group of animals (n=5) was injected with [I-125]-Z-(R,R)-IQNP. The rats were anesthetized and euthenized by cervical dislocation 3 hours post injection of [I-125]-Z-(R,R)-IQNP. The various organs were removed, rinsed with saline, blotted dry and weighed in tared vials. Blood samples were obtained from the thoracic cavity after removal of the heart. The brains were removed, placed on a cold surface (ice) and dissected into the various regions immediately upon removal. The tissues were weighed and then counted in a Packard Minaxi 5000 sodium iodide auto gamma counter.

For dual-isotope studies, ethanolic solutions (100 μ L) of [I-131]-E- and [I-125]-Z-(R,R)-IQNP were mixed in a vial, 0.1 N HCl solution (100 μ L) added and the volume brought up to 10 mL with normal saline. Following filtration through a 0.22 μ Millipore filter, the solution was administered by intravenous injection (0.5 mL) into a lateral tail vein of metophane-anesthetized rats (n=5 rats/group, I-125: 4-6 μ Ci; I-131: 8-12 μ Ci). The tissue dissection was performed as above. After weighing, the tissues were first counted for I-131, placed in the freezer for one month and then counted for I-125.

For triple-isotope studies, ethanolic solutions of [I-125]-4IQNB (100 μ L), [I-131]-IDEX (200 μ L) and [I-123]-Z-(R,R)-IQNP (100 μ L) were mixed in a vial, ethanol (0.6 mL) and 0.1 N HCl (0.1 mL) added and the resultant solution filtered through a 0.22 μ Millipore filter into an injection vial and the filter was then washed with saline (10 mL) into the injection vial. The resultant solution was administered by intravenous injection (0.5 mL) into a lateral tail vein of groups metophane-anesthetized rats (n=5 rats/group, [I-125]-4IQNB: 5.5 μ Ci, [I-131]-IDEX: 10.1 μ Ci, [I-123]-Z-(R,R)-IQNP: 5.3 μ Ci). The tissue dissection was performed as above. After weighing, the tissues were initially counted for I-123, followed by I-131 after 7 days and I-125 after 42 days. The tissues were stored in the freezer during the time between counting.

Tissue Extraction and Analysis Tissue samples were extracted by the traditional Folch technique as described previously, using a loose fitting Potter-Elmehahn ground glass homogenizer (34). For global brain and heart tissues, the chilled organs were finely minced in a watch glass and then homogenized for 3 minutes in a chloroform:methanol solution (10 mL, 2:1 v/v). Blood samples were obtained by cardiac puncture prior to removal of the heart. Blood samples were taken up in a solution of a saturated EDTA solution (0.1 mL) and centrifuged to separate plasma from the pellet. The plasma fraction was then homogenized and extracted as above. Similar treatment of the cellular pellets by this procedure resulted in an insoluble sticky mass that could not be analyzed. The extracts were filtered through filter paper into centrifuge tubes and the filters washed with chloroform (1 mL). The filtrates were thoroughly mixed with normal saline (2 mL) and centrifuged at low speed for 10 minutes to separate the organic and aqueous layers. The organic layer was carefully removed with a pipette, filtered through a short column of anhydrous sodium sulfate and evaporated to dryness under a stream of argon. The resulting organic and aqueous fractions and the filter paper containing the pellet were counted. The organic residues were dissolved in chloroform (0.5 mL) and analyzed for Z-(R,R)-IQNP by TLC analysis (chloroform:methanol [8.5:1.5], $R_f=0.40$). Selected organic fractions from the heart and brain were also analyzed by HPLC after dissolution in HPLC solvent (1.0 mL) and addition of a standard sample of Z-(R,R)-IQNP.

Urine and Fecal Analysis A group of rats ($n=5$) were housed individually in separate metabolism cages and urine and feces were collected daily for 7 days. Samples of the urine from day 1 and 2 were applied to TLC plates and analyzed by TLC with a mobile phase of chloroform:acetic acid (7:3).

Results

[I-125]-Z-(R,R)-IQNP Blocking Study The ability of various established receptor-specific antagonists to block the uptake of Z-(R,R)-IQNP in cerebral regions and the heart was evaluated. Groups of rats were treated with either QNB, SCO, DEX (subtype nonselective mAChR antagonists), NAL (opiate receptor antagonist), (-)-BUT (sigma antagonist) and (+)-BUT (D_1/D_2 dopamine antagonist) prior to the injection of [I-125]-Z-(R,R)-IQNP. The rats were killed 3 hours post injection of [I-125]-Z-(R,R)-IQNP. It was observed that treatment with QNB, SCO or DEX effectively blocked the cerebral and cardiac uptake of activity in all regions investigated (Figure 2). In contrast, treatment with (-)- BUT, (+)-BUT or NAL at the dosage administered demonstrated no effect on the distribution of cerebral and cardiac radioactivity in comparison to controls.

[I-131]-E / [I-125]-Z-(R,R)-IQNP Dual-Labeled Biodistribution Study The direct *in vivo* comparison of the biodistribution of [I-125]-Z-(R,R)-IQNP and [I-131]-E-(R,R)-IQNP was performed and the results are shown in Table 1. This study was performed by the administration of a solution of E-/Z-(R,R)-IQNP in the same animal. By allowing each animal to be its own control, subtle differences in the biodistribution of the two stereoisomers of IQNP were evaluated. Z-(R,R)-IQNP demonstrated significantly greater uptake in cerebral regions containing a high concentration of m_2 mAChR subtype at 6 hours post injection as compared to E-(R,R)-IQNP. The uptake of activity in other cerebral regions and the heart was also observed to be greater for Z-(R,R)-IQNP as compared to E-(R,R)-IQNP. In addition, the level of activity observed in the liver was significantly less for the Z-isomer. In other tissues investigated (kidney, lung, blood) the uptake of activity was similar to that reported previously (28).

[I-131]-IDEX / [I-125]-4IQNB / [I-123]-Z-(R,R)-IQNP Triple-Labeled Biodistribution Study Analogous to the study discussed above, a direct comparison of the biodistribution of [I-131]-IDEX, [I-125]-4IQNB and [I-123]-Z-(R,R)-IQNP was performed in the same animal over 4 hours (Table 2). It was observed at 4 hours, uptake of Z-(R,R)-IQNP was significantly greater as compared to IDEX and 4IQNB in cerebral regions containing a high concentration of m2 mAChR subtype. The uptake of activity associated with Z-(R,R)-IQNP in other tissues investigated demonstrated favorable imaging properties (Table 3).

Ex Vivo Metabolism Study An *ex vivo* metabolism study was performed to evaluate the radioactive species that crosses the blood-brain-brain barrier and binds at the receptor site (Table 4). In global brain tissue, approximately 80% of the activity was extracted into the organic phase, the remaining activity associated with the pellet and filter. The activity in the organic phase was observed to be greater than 97% unmetabolized Z-(R,R)-IQNP by TLC and HPLC analysis up to 24 hours. In global heart tissue, approximately 75% of the activity was extracted into the organic phase, 20% in the pellet and filter and 5% in the aqueous fraction up to 4 hours. The activity in the organic phase was observed to be greater than 97% unmetabolized Z-(R,R)-IQNP by TLC and HPLC analysis. However, by 24 hours, a decrease in the activity extracted into the organic phase, in addition to the increase of metabolites as determined by TLC analysis, was observed in heart tissue.

Analysis of the plasma demonstrated that approximately 8% of the activity was extracted into the organic phase at 60 min post injection and the activity identified as Z-(R,R)-IQNP by TLC decreased from approximately 80% at 30 minutes to approximately 15% at 24 hours with the activity remaining at the origin of the TLC plate. It was also observed that the major pathway for the excretion for Z-(R,R)-IQNP was through the urine (approximately 60%) and the radioactivity was effectively cleared from the rat by the third day (Table 5).

Discussion

We have recently developed a new quinuclidinyl ester, IQNP, for the potential non-invasive imaging of mAChR by SPECT. Initial *in vivo* biodistribution studies in female rats utilizing the Z-(R,R)-isomer have demonstrated a significant cerebral uptake in regions which contain a high concentration of the m2 mAChR subtype (cerebellum, brain stem, thalamus, colliculi's) (2) in addition to the heart (28). To evaluate if the observed activity in these regions was receptor mediated uptake and not due to nonspecific binding, a blocking study was performed utilizing Z-(R,R)-IQNP and a series of established receptor specific antagonists. Since there are no established ligands that demonstrate a high selectivity for binding at the m2 mAChR subtype and penetrate the blood brain barrier, a series of established non-selective ligands were employed for this study. The ligands utilized were scopolamine (SCO), (R)-3-quinuclidinyl benzilate (R-QNB) and dexetimide (DEX). In addition, a selection of ligands which do not display affinity to mAChR were also investigated. [(+)-butaclamol ((+)-BUT, D₁/D₂ dopamine antagonist), (-)-butaclamol ((-)-BUT, sigma antagonist) and naloxone (NAL, opiate antagonist)]. The results of this study demonstrate R-QNB, SCO and DEX block the cerebral and cardiac uptake of activity demonstrating that Z-(R,R)-IQNP non-selectivity binds to mAChR and the uptake observed in m2 rich areas is due to binding of the ligand at the receptor site (Figure 2). The other blocking agents utilized in this study failed to block the uptake of Z-(R,R)-IQNP in these same regions and tissues. Therefore this study demonstrates Z-(R,R)-

IQNP binds to the receptor site in cerebral and cardiac regions containing a high concentration of the m2 mAChR subtype and the observed uptake and retention of activity is not indicative of nonspecific binding of the radioligand in these regions.

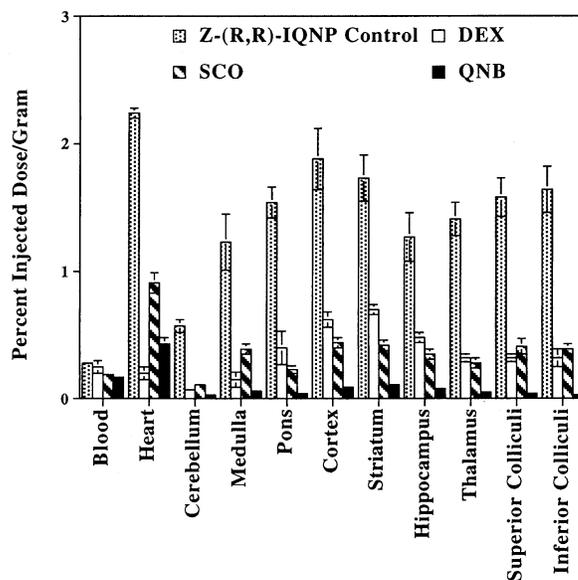


Fig. 2 Blocking study of Z-(R,R)-IQNP with pretreatment of DEX, SCO or QNB in female rats (n=5).

Iodine has three commercially available isotopes whose unique decay properties and specific activities (SA) are suitable for *in vivo* receptor labeling studies (I-125: $t_{1/2}$ =60 days, 27 KeV, SA 17.4 Ci/mg; I-131: $t_{1/2}$ =8 d, 364 KeV, SA 7.7 Ci/mg; I-123: $t_{1/2}$ =13.2 h, 140 KeV, SA 1900 Ci/mg). By utilizing the decay energy and half-life of each isotope, it is possible to perform a direct *in vivo* comparison study in the same animal allowing each animal to act as its own control. Utilizing this technique, the effects of age, injection technique, circadian rhythm and diet, for example, do not distort the data and actual binding differences can be evaluated.

Previous *in vitro* and *in vivo* evaluation utilizing E- and Z-(R,R)-IQNP in separate groups of rats demonstrated a difference in the biological properties of these ligands. In these studies, E-(R,R)-IQNP showed high binding to the m1, m4 mAChR subtypes after time was allowed for the modest binding to m2 subtype to clear and Z-(R,R)-IQNP showed high binding in all cerebral regions and the heart indicating a non-subtype selective uptake (28,29). A dual label study utilizing [I-125]-Z-(R,R)-IQNP and [I-131]-E-(R,R)-IQNP was performed to evaluate these subtle differences in the *in vivo* subtype binding of each isomer in the same animal (Table 1). From the results of this study, it is important to note the calculated percent injected dose/gram (%ID/G) from this study and from studies utilizing the single isomers is similar (28). Therefore the dual-labeled E-/Z-(R,R)-IQNP injection solution has sufficiently high specific activity and the resultant mass does not compete with the radiolabeled ligands for mAChR binding sites. In this study, an actual measured difference in the uptake of the two isomers in the various regions of the brain, in addition to the heart and liver, was observed. Z-(R,R)-IQNP was observed to have a higher uptake and longer retention in tissues that contain a high

concentration of the m2 mAChR subtype (cerebellum, pons, medulla, thalamus and colliculi's) as directly compared to E-(R,R)-IQNP.

Table 1. Dual Label *In Vivo* Biodistribution Evaluation of [I-131]-E-(R,R)-IQNP and [I-125]-Z-(R,R)-IQNP in Female Rats (n=5).

Organ	Isomer	Percent Injected Dose/Gram					
		15 min	30 min	60 min	120 min	240 min	360 min
Cerebellum	E-	0.59±0.08	0.48±0.06	0.25±0.02	0.13±0.01	0.07±0.02	0.04±0.02
	Z-	0.87±0.09	0.96±0.13	0.75±0.07	0.52±0.05	0.35±0.09	0.23±0.01
Pons	E-	1.10±0.31	1.32±0.38	1.27±0.36	1.09±0.15	0.77±0.23	0.58±0.17
	Z-	1.36±0.40	1.75±0.49	1.52±0.24	1.59±0.22	1.32±0.27	1.33±0.23
Medulla	E-	0.94±0.09	0.91±0.17	0.82±0.14	0.59±0.11	0.39±0.09	0.26±0.03
	Z-	1.33±0.07	1.24±0.16	1.10±0.19	1.06±0.08	1.04±0.08	0.80±0.07
Cortex	E-	1.49±0.11	1.91±0.37	1.72±0.23	1.69±0.23	1.49±0.12	1.68±0.12
	Z-	1.36±0.09	1.77±0.31	1.69±0.26	1.73±0.23	1.55±0.16	1.86±0.15
Striatum	E-	1.32±0.18	1.73±0.32	1.60±0.27	1.53±0.17	1.43±0.26	1.51±0.16
	Z-	1.28±0.12	1.73±0.27	1.61±0.25	1.62±0.19	1.54±0.25	1.66±0.18
Hippocampus	E-	1.00±0.10	1.30±0.24	1.22±0.14	1.17±0.10	1.03±0.11	1.19±0.11
	Z-	0.98±0.10	1.22±0.25	1.16±0.16	1.24±0.11	1.11±0.12	1.34±0.12
Thalamus	E-	1.21±0.10	1.44±0.38	1.28±0.19	1.15±0.11	0.98±0.20	0.78±0.04
	Z-	1.26±0.15	1.53±0.30	1.43±0.18	1.42±0.16	1.29±0.14	1.33±0.06
Superior Colliculi	E-	1.13±0.10	1.35±0.29	1.08±0.21	0.84±0.13	0.65±0.16	0.47±0.09
	Z-	1.28±0.14	1.63±0.37	1.42±0.31	1.43±0.17	1.38±0.09	1.36±0.16
Inferior Colliculi	E-	1.17±0.15	1.38±0.25	1.07±0.05	0.87±0.08	0.62±0.17	0.42±0.03
	Z-	1.46±0.15	1.78±0.32	1.58±0.11	1.55±0.17	1.43±0.22	1.31±0.11
Rest of Brain	E-	1.02±0.10	1.27±0.22	1.14±0.15	0.98±0.16	0.84±0.11	0.87±0.06
	Z-	1.01±0.10	1.29±0.21	1.24±0.16	1.20±0.16	1.07±0.11	1.19±0.08

R-3-Quinuclidinyl S-4-iodobenzilate (4IQNB) and iododexetimide (IDEX) have been utilized as probes for the evaluation of mAChR in healthy individuals and patients with various dementias with SPECT (35-43). Although 4IQNB and IDEX do not demonstrate mAChR subtype selectivity, they have been utilized to study mAChR in frontal, temporal, parietal and occipital cortex and neostriatum. However, these ligands have not shown potential for the study of cerebral regions which contain a high concentration of the m2 subtype (brain stem, cerebellum, thalamus) and the heart due to the short residence time of the ligand at the m2 receptor site and the prolonged time required between administration and imaging required to allow nonspecific binding to clear.

A triple label study was performed to directly compare the *in vivo* biodistribution of Z-(R,R)-IQNP, IDEX and 4IQNB. IDEX, 4IQNB and Z-(R,R)-IQNP were labeled with I-131, I-125 and I-123, respectively, as previously reported (28,32,33). As discussed above, by comparing the %ID/G in Tables 1 and 2, the injection solution containing the three radioiodinated ligands affords a solution of sufficient SA for direct *in vivo* comparison of the binding of these ligands. In this study, Z-(R,R)-IQNP demonstrated higher uptake in the various regions of the brain and heart as directly compared to IDEX and 4IQNB (Tables 2 and 3). In addition, Z-(R,R)-IQNP demonstrated higher uptake and longer retention time in regions of the brain which contain a high concentration of the m2 subtype. It was also observed that the radioactivity associated with Z-(R,R)-IQNP that localized in non-target organs (liver, kidney, lung) washed out faster as compared to IDEX and 4IQNB. Due to its higher cerebral

uptake, a smaller dose may be required for imaging studies decreasing the cost and minimizing the radiation dose to the patients and medical personnel. In addition, Z-(R,R)-IQNP has the potential to image regions that contain a high concentration of the m2 mAChR subtype (heart, brain stem, cerebellum).

Table 2. Triple Labeled Study of the Cerebral Distribution of [I-123]-Z-(R,R)-IQNP, [I-131]-IDEX and [I-125]-(R,S)-4IQNB in Female Rats (n=5).

Organ	Compound	Percent Injected Dose/Gram		
		15 Minutes	120 Minutes	240 Minutes
Cerebellum	Z-(R,R)-IQNP	0.84 ± 0.16	0.70 ± 0.17	0.39 ± 0.05
	IDEX	0.33 ± 0.05	0.17 ± 0.03	0.12 ± 0.00
	R,S-4IQNB	0.43 ± 0.08	0.18 ± 0.05	0.08 ± 0.00
Pons	Z-(R,R)-IQNP	1.39 ± 0.46	1.81 ± 0.49	1.40 ± 0.11
	IDEX	0.76 ± 0.19	0.83 ± 0.22	0.52 ± 0.07
	R,S-4IQNB	0.81 ± 0.28	1.04 ± 0.38	0.65 ± 0.10
Medulla	Z-(R,R)-IQNP	1.01 ± 0.15	1.38 ± 0.31	1.04 ± 0.22
	IDEX	0.45 ± 0.09	0.35 ± 0.09	0.18 ± 0.02
	R,S-4IQNB	0.58 ± 0.11	0.59 ± 0.15	0.37 ± 0.09
Cortex	Z-(R,R)-IQNP	1.47 ± 0.31	1.88 ± 0.36	2.05 ± 0.25
	IDEX	1.22 ± 0.25	1.28 ± 0.22	1.05 ± 0.05
	R,S-4IQNB	1.08 ± 0.29	1.40 ± 0.33	1.45 ± 0.20
Striatum	Z-(R,R)-IQNP	1.11 ± 0.42	1.60 ± 0.29	1.61 ± 0.49
	IDEX	0.96 ± 0.36	1.25 ± 0.25	0.91 ± 0.27
	R,S-4IQNB	0.84 ± 0.37	1.22 ± 0.25	1.15 ± 0.32
Hippocampus	Z-(R,R)-IQNP	1.02 ± 0.18	1.35 ± 0.30	1.32 ± 0.18
	IDEX	0.85 ± 0.13	1.14 ± 0.23	0.94 ± 0.08
	R,S-4IQNB	0.70 ± 0.14	1.00 ± 0.27	0.93 ± 0.19
Thalamus	Z-(R,R)-IQNP	1.29 ± 0.27	1.63 ± 0.36	1.51 ± 0.23
	IDEX	0.80 ± 0.15	0.77 ± 0.14	0.50 ± 0.02
	R,S-4IQNB	0.86 ± 0.19	1.11 ± 0.30	0.91 ± 0.16
Superior Colliculi	Z-(R,R)-IQNP	1.39 ± 0.28	1.67 ± 0.54	1.42 ± 0.28
	IDEX	0.75 ± 0.13	0.55 ± 0.15	0.38 ± 0.02
	R,S-4IQNB	0.83 ± 0.17	0.87 ± 0.29	0.54 ± 0.10
Inferior Colliculi	Z-(R,R)-IQNP	1.50 ± 0.26	1.88 ± 0.52	1.40 ± 0.23
	IDEX	0.71 ± 0.11	0.52 ± 0.16	0.32 ± 0.05
	R,S-4IQNB	0.80 ± 0.21	0.87 ± 0.29	0.54 ± 0.05

Since high uptake of radioactivity in the liver and lung in relationship to the heart can interfere with imaging studies of the heart, Z-(R,R)-IQNP was observed to demonstrate a favorable heart to liver (1.2:1) and heart to blood ratio (2.9:1) as compared to IDEX (0.4:1, 1.5:1) and 4IQNB (0.5:1, 2.3:1), respectively, at 4 hours (Table 3).

An initial *ex vivo* metabolic study over 24 h directed at the determination of the radioactive species in global brain and heart tissue post injection of Z-(R,R)-IQNP was performed (Table 4). Tissue Folch extracts of the global brain demonstrated the lipid-soluble extracts contained approximately 80% of the activity, the aqueous fractions contained 1% of the activity with the remainder of the activity associated with the pellet and filter paper. The lipid-soluble brain extract indicated the presence of only unmetabolized Z-(R,R)-IQNP by TLC and HPLC analysis over the course of this study.

Table 3. Triple Labeled Study in Various Tissues of [I-123]-Z-(R,R)-IQNP, [I-131]-IDEX and [I-125]-4IQNB in Female Rats (n=5).

Organ	Compound	Percent Injected Dose/Gram (\pm SD)		
		15 Minutes	120 Minutes	240 Minutes
Blood	Z-(R,R)-IQNP	0.41 \pm 0.02	0.32 \pm 0.04	0.30 \pm 0.03
	IDEX	0.18 \pm 0.01	0.30 \pm 0.02	0.39 \pm 0.03
	R,S-4IQNB	0.19 \pm 0.01	0.13 \pm 0.01	0.10 \pm 0.01
Liver	Z-(R,R)-IQNP	1.27 \pm 0.06	0.83 \pm 0.07	0.74 \pm 0.02
	IDEX	3.26 \pm 0.17	2.23 \pm 0.37	1.54 \pm 0.13
	R,S-4IQNB	1.86 \pm 0.12	0.81 \pm 0.17	0.45 \pm 0.04
Kidney	Z-(R,R)-IQNP	1.40 \pm 0.07	0.86 \pm 0.09	0.56 \pm 0.12
	IDEX	1.78 \pm 0.14	1.27 \pm 0.22	0.94 \pm 0.06
	R,S-4IQNB	2.63 \pm 0.25	1.14 \pm 0.23	0.57 \pm 0.07
Heart	Z-(R,R)-IQNP	5.11 \pm 0.66	2.17 \pm 0.20	0.86 \pm 0.13
	IDEX	1.32 \pm 0.06	0.87 \pm 0.08	0.59 \pm 0.06
	R,S-4IQNB	1.43 \pm 0.12	0.55 \pm 0.06	0.23 \pm 0.02
Lung	Z-(R,R)-IQNP	5.13 \pm 0.95	1.65 \pm 0.24	0.77 \pm 0.06
	IDEX	6.04 \pm 0.78	3.43 \pm 0.61	1.97 \pm 0.22
	R,S-4IQNB	11.68 \pm 1.76	3.45 \pm 0.69	1.28 \pm 0.16

The lipid-soluble fraction from Folch extracts of heart tissue contained approximately 75% of the activity. In contrast to that observed in brain tissue, the aqueous fraction contained approximately 6% of the activity with the remainder of the activity contained in the pellet and filter paper up during the first 4 hours of the study. At 24 hours post injection, the fraction of activity extracted in the aqueous solution increased to 15%. TLC analysis of the lipid-soluble heart extract indicated the presence of only unmetabolized Z-(R,R)-IQNP up to 6 hours post injection and declining to approximately 94% at 24 hours (Table 4).

Table 4. *Ex Vivo* Metabolic Profile of [I-125]-Z-(R,R)-IQNP in Female Rats (n=5).

Organ	Time (min)	Percent Activity (\pm SD)			Percent IQNP In Organic Phase
		Aqueous Phase	Pellet/Filter	Organic Phase	
Brain	30	0.9 \pm 0.4	18.4 \pm 2.2	80.9 \pm 2.1	98.8 \pm 0.7
	60	1.2 \pm 2.0	20.3 \pm 1.5	78.2 \pm 2.5	98.5 \pm 1.7
	240	0.7 \pm 0.3	19.8 \pm 5.6	79.3 \pm 5.4	98.2 \pm 0.6
	1440	0.6 \pm 0.2	20.4 \pm 2.3	79.1 \pm 2.4	97.6 \pm 0.8
Heart	30	5.2 \pm 0.5	19.8 \pm 3.7	75.0 \pm 3.6	98.1 \pm 0.9
	60	6.1 \pm 1.4	17.3 \pm 1.5	76.7 \pm 2.0	98.3 \pm 0.2
	240	5.7 \pm 1.4	21.7 \pm 1.8	72.6 \pm 1.1	98.7 \pm 0.5
	1440	15.4 \pm 2.1	22.9 \pm 2.3	61.7 \pm 1.5	94.1 \pm 2.5
Plasma	30	36.7 \pm 17.0	55.4 \pm 15.0	7.9 \pm 2.7	79.4 \pm 11.2
	60	40.4 \pm 14.8	54.6 \pm 13.9	5.0 \pm 1.2	74.0 \pm 6.2
	240	48.2 \pm 8.1	50.4 \pm 8.3	1.4 \pm 0.4	55.6 \pm 10.8
	1440	38.3 \pm 14.0	56.9 \pm 14.3	4.8 \pm 1.5	16.9 \pm 10.7

In contrast, the plasma lipid-soluble organic fraction from Folch extracts contained approximately 5% of the activity (Table 4). In addition, the amount of Z-(R,R)-IQNP observed

in the plasma by TLC decreased from 79% at 30 minutes to 17% at 24 hours post injection with the subsequent increase in the formation of a polar metabolite as determined by TLC analysis.

A group of animals were housed in metabolism cages to analyze the excretion of activity after injection of Z-(R,R)-IQNP. It was observed approximately 70% of the injected activity was excreted by the fourth day with higher urinary excretion (51.2%) as compared to the feces (21.4%) (Table 5). An absence a unmetabolized Z-(R,R)-IQNP in the urine was observed by TLC analysis.

Table 5. Excretion Profile of [125 I]-Z-(R,R)-IQNP in Female Rats (n=5).

Time (days)	Percent Activity Excreted (\pm SD)		Cumulative Activity Excreted (\pm SD)
	Urine	Feces	
1	40.4 \pm 4.9	13.4 \pm 1.0	53.8 \pm 5.0
2	7.4 \pm 0.8	5.4 \pm 1.2	66.6 \pm 5.2
3	2.2 \pm 0.4	1.7 \pm 1.7	70.4 \pm 5.2
4	1.2 \pm 0.4	0.9 \pm 0.2	72.5 \pm 5.2
5	1.0 \pm 0.2	0.5 \pm 0.1	74.0 \pm 5.2
6	1.0 \pm 0.0	0.4 \pm 0.2	75.4 \pm 5.2
7	0.5 \pm 0.0	0.2 \pm 0.0	76.0 \pm 5.2

Conclusion

The *in vivo* biological property of Z-(R)-1-azabicyclo[2.2.2]oct-3-yl (R)- α -hydroxy- α -(1-iodo-1-propen-3-yl)- α -phenylacetate (Z-(R,R)-IQNP), was evaluated for potential use for imaging cerebral and cardiac m2 subtype rich regions. *In vivo* blocking study with a series of established nonselective mAChR specific antagonists demonstrated a significant binding of Z-(R,R)-IQNP to cerebral and cardiac mAChR regions containing a high concentration of the m2 subtype. A dual label study, allowing the direct comparison of the *in vivo* binding of E- and Z-(R,R)-IQNP in the same animal, demonstrated a significantly higher uptake and longer retention time of Z-(R,R)-IQNP in cerebral and cardiac regions containing a high concentration of the m2 mAChR subtype. An analogous triple label study utilizing Z-(R,R)-IQNP, IDEX and 4IQNB was also performed. Z-(R,R)-IQNP was observed to have higher uptake and longer residence time at m2 rich mAChR cerebral and cardiac regions and faster washout from non-target organs. *Ex vivo* metabolic studies demonstrated that Z-(R,R)-IQNP was the sole component identified from lipid soluble fractions of global brain and heart tissues. The results of these studies demonstrate that Z-(R,R)-IQNP, with its high uptake, long retention and metabolic stability is an attractive ligand for the imaging m2 rich areas of the brain and heart. These attractive properties may also allow same day imaging of m2 rich mAChR regions allowing for a better understanding of the normal aging process in addition to an earlier diagnosis and cost effective treatment of various diseases and dementias.

Acknowledgments

The authors thank Dr. B. Zeeberg for the gift of the tributyltin precursor for the labeling of 4IQNB and Dr. B. Maziere for the gift of the trimethylsilyl precursor for the labeling of IDEX. Research was supported at Oak Ridge National Laboratory by the Office of Science, U. S. Department of Energy, under contract DE-AC05-96OR22464 with Lockheed Martin Energy Research Corporation.

References

1. N.J. BUCKLEY, T.I. BONNER, C.M. BUCKLEY and M.R. BRANN, *Mol. Pharmacol.* **35** 469-476 (1989).
2. A. I. LEVEY, *Life Sci.* **52** 441-448 (1993).
3. A. NORDBERG, I. ALAGUZOFF and B. WINBLAD, *J. Neurosci. Research* **31** 103-111(1992).
4. H.C. KINNEY, J.J. FILIANO, L.A. SLEEPER, L.F. MANDEL, M. VALDES-DAPENA and W.F. WHITE, *Science* **269** 446-1450 (1995).
5. K. TANAKA, N. OGAWA, M. ASANUMO, Y. KONDO and M. NOMURA, *Brain Res.* **729** 55 (1996).
6. A. I. LEVEY, *Proc. Natl. Acad. Sci. USA* **93** 13541-13546 (1996).
7. R.J. SALIN-PASCUAL, D. GRANADOS-FUENTES, L. GALICIA-POLO and E. NIEVES, *Neuropsychopharmacology* **5** 183-186 (1991).
8. G. FREUND and W.E. BALLINGER, *Alcohol & Alcoholism Suppl.* **1** 385-391 (1991).
9. E. HELLSTROM-LINDAHL, B. WINBLAD and A. NORDBERG, *Brain Res.* **620** 42-48 (1993).
10. I. AUBERT, D. M. ARAUJO, D. CECYRE, Y. ROBITAILLE, S. GAUTHIER, R. QUIRION, *J. Neurochem.* **58** 529-541 (1992).
11. D.D. FLYNN, G. FERRARI-DILEO, D.C. MASH and A.I. LEVEY, *J. Neurochem.* **64** 1888-1891 (1995).
12. S. JENNI-EIERMANN, H.P. VON HAHN, G.C. HONEGGER and J. ULRICH, *Gerontology* **30** 350-358 (1984).
13. M. MCKINNEY and J.T. COYLE, *Mayo Clin. Proc.* **66** 1225-1237 (1991).
14. E. PERRY, *Br. J. Psychiat.* **152** 737-740 (1988).
15. R. QUIRION, I. AUBERT, P.A. LAPCHAK, R.P. SCHAUM, S. TEOLIS, S. GAUTHIER and D. M. ARAUJO, *Trends Pharmacol. Sci. Suppl.* **IV** 80-84 (1989).
16. G.-J. WASTEK and H.I. YAMAMURA, *Mol. Pharmacology* **14** 768-780 (1978).
17. D.C. MASH, D.D. FLYNN and L.T. POTTER, *Science* **228** 1115-1117 (1985).
18. K. ZILLES, M. QU, A. SCHLEICHER, M. SCHROETER, M. KRAEMER and O.W. WITTE, *Arzneim-Forsch/Drug Res.* **45** 361-366 (1995).
19. H. YAMAGUCHI, S. HIRAI, M. MORIMATSU, M. SHOJI and Y. NAKAZATO, *Acta. Neuropathol.* **77** 314-319 (1989).
20. G. COLE, J.W. NEAL, S.K. SINGHRAO, B. JASANI and G.R. NEWMAN, *Acta. Neuropathol.* **85** 542-552 (1993).
21. H.A. BAGHDOYAN, R. LYDIC, M.A. FLEEGAL, *J. Pharmacol. Exp. Ther.* **286** 1446-1452 (1998).
22. H.A. BAGHDOYAN, *Sleep Science: Integrating Basic Research and Clinical Practice. Monographs in Clinical Neuroscience*, WJ Schwartz ed, **15**, 88-116, Karger, Basel (1997).
23. M. STERIADE, R.W. MCCARLEY, *Brain Stem Control of Wakefulness and Sleep*, Plenum Press, New York, NY (1990).
24. J.Z. FIELDS, W.R. ROESKE, E. MORKIN and H.I. YAMAMURA, *J. Biol. Chem.* **253** 3251-3258 (1978).
25. J.C. GOIN, E. BORDA, C.P. LEIROS, R. STORINO and L. STERIN-BORDA, *J. Auton. Nerv. Sys.* **47** 45-52 (1994).
26. M.L.X. FU, *Eur. Heart J.* **16** 89-91 (1995).
27. D.W. MCPHERSON, D.L. DEHAVEN-HUDKINS, A.P. CALLAHAN and F.F. KNAPP, Jr., *J. Med. Chem.* **36** 848-854 (1993).
28. D.W. MCPHERSON, C.R. LAMBERT, K. JAHN, V. SOOD, R.C. MCCREE, B. ZEEBERG, R.C. REBA and F.F. KNAPP, Jr., *J. Med. Chem.* **38** 3908-3917 (1995).

29. M.R. RAYEQ, S.F. BOULAY, V.K. SOOD, D.W. MCPHERSON, F.F. KNAPP, Jr., B. ZEEBERG and R.C. REBA, *Receptors and Signal Transduction* **6** 13-34 (1996).
30. M.S. GITLER, S.F. BOULAY, V.S. SOOD, D.W. MCPHERSON, F.F. KNAPP, Jr., B.R. ZEEBERG and R.C. REBA, *Brain Research* **687** 71-78 (1995).
31. W.J. RZESZOTARSKI, D.W. MCPHERSON, J.W. FERKANY, W.J. KINNIER, L. NOROHA-BLOB and A. KIRKEN-RZESZOTARSKI, *J. Med. Chem.* **31** 1463-1466 (1988).
32. K.S. LEE, X.S. HE, D.W. JONES, R. COPPOLA, J.G. GOREY, M.B. KNABLE, B.R. DECOSTA, K.C. RICE and D.R. WEINBERGER, *J. Nucl. Med.* **37** 2021-2024 (1996).
33. A.A. WILSON, R.F. DANNALS, H.T. RAVERT, J.J. FROST and H.N. WAGNER, Jr., *J. Med. Chem.* **32** 1057-1062 (1989).
34. D.W. MCPHERSON, C.R. LAMBERT and F.F. KNAPP, Jr., *Eur. J. Nucl. Med.* **21** 1293-1297 (1994).
35. D.R. WEINBURGER, R. GIBSON, R. COPPOLA, D.W. JONES, S. MOLCHAN, T. SUNDERLAND, K.F. BERMAN and R.C. REBA, *Arch. Neurol.* **48** 169-176 (1991).
36. D.R. WEINBURGER, D. JONES, R.C. REBA, R.C. MANN, R. COPPOLA, R. GIBSON, J. GOREY, S.A. BRAUN, and T.N. CHASE, *J. Neuropsychiatry Clinical. Neurosci.* **4** 239-248 (1992).
37. J. WYPER, D. BROWN, J. PATTERSON, J. OWENS, R. HUNTER, E. TEASDALE, and J. MCCULLOCH, *Eur. J. Nucl. Med.* **20** 379-386 (1993).
38. J.J. CLAUS, E.A. DUBOIS, J. BOOIJ, J. HABRAKEN, J.C. DE MUNCK, M. VAN HERK, B. VERBEETEN and E.A. VAN ROYEN, *Eur. J. Nucl. Med.* **24** 602-608 (1997).
39. E.A. DUBOIS, J. CLAUS, J. BOOIJ, J. HABRAKEN, J.C. DE MUNCK, B.W.J.M. VERBEETEN, G.J.M. WALSTRA, E.A. VAN ROYEN, M. PFAFFENDORF and P.A. VAN ZWIETEN, *Life Sci.* **60** 85 (1997).
40. H.W. MULLER-GARTNER, A.A. WILSON, R.F. DANNALS, H.N. WAGNER, Jr., and J.J. FROST, *J. Cere. Blood Flow Metab.* **12** 562-570 (1992).
41. H.W. MULLER-GARTNER, R.S. FISHER, R.P. LESSER, A.A. WILSON, H.T. RAVERT, R.F. DANNALS, H.N. WAGNER, Jr., and J.J. FROST, *Eur. J. Nucl. Med.* **19** 736 (1992).
42. H.W. MULLER-GARTNER, H.S. MAYBERG, R.S. FISHER, R.P. LESSOR, A.A. WILSON, H.T. RAVERT, R.F. DANNALS, H.N. WAGNER, Jr., S. UEMATSU and J.J. FROST, *Ann. Neurol.* **34** 235-238 (1993).
43. M. ECKESSER, A. HUFNAGEL, K. ZIEMONS, M. GRIESSMEIER, F. SONNENBERG, T. HACKLANDER, K.J. LANGEN, M. HOLSCHBACH, C.E. ELGER and H.W. MULLER-GARTNER, *Eur. J. Nucl. Med.* **24** 1156-1161 (1997).