Use of 3-Quinuclidinyl 4-Iodobenzilate as a Receptor Binding Radiotracer

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3-Quinuclidinyl 4-iodobenzilate was shown to bind to the muscarinic acetylcholine receptor (mAChR) by testing the saturability and the stereoselectivity in the corpus striatum, cerebellum, and the heart. But the ratio of radioactivity in tissues containing different concentrations of mAChR was less than the ratio of mAChR concentrations determined by in vitro saturation assay. As a result, the sensitivity to change in receptor concentration by external imaging will be reduced for this receptor binding radiotracer.

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There are two important criteria that must be fulfilled before potential receptor binding radiotracers can be used to measure changes in receptor concentration as a function of disease. The first is realized by testing the operational definition of the receptor to prove that the radioactivity is bound to the receptor. The second is satisfied by measuring the change in radioactivity bound to the receptor as a function of the receptor concentration.

Both of these criteria were tested in order to determine if 3-quinuclidinyl 4-iodobenzilate (4-IQNB), a muscarinic acetylcholine receptor (mAChR) binding radiotracer, can be used in vivo to measure the change in muscarinic receptor concentration that may occur in disease states.

MATERIALS AND METHODS

All radioiodinated ligands were prepared according to the method of Rzeszotarski et al. (*1*). The iodinated QNB analog (4-[^{125}I] IQNB) was synthesized by the reaction of iodine with the 4-triazeno derivative of QNB with an overall yield of 20%. The product, isolated by reversed-phase high performance liquid chromatography (HPLC), had an elution volume identical with that of an authentic sample of stable 4-IQNB (analysis within 0.4% of the theoretical). There is no uv peak coincident with the peak of radioactivity; nonetheless, the specific activity of the 4-[^{125}I]IQNB used in these studies varied from 900–1200 Ci/mmol. Trailing uv-absorbing peaks that elute before the desired product are the most probable cause of the reduced specific activity. Radiochemical purity was determined by thin layer chromatography (TLC) (95%); the product exhibited an R_f value consistent with the authentic sample: $R_f = 0.35$ in toluene/MeOH (80:20) and = 0.5 in butanol/acetic acid/-water (4:1:1). The stereoisomers used for this study and previous studies are shown in Table 1. R-[^{3}H]QNB was obtained commercially.*

Animal studies

The in vivo distribution was determined in male Sprague-Dawley rats weighing 250–300 g at the time of study. The thyroid was not blocked. Under light halothane anesthesia, 0.1 ml of radiopharmaceutical solution (3–5 μCi in 50% ethanol) was injected into an exposed femoral vein. At selected times after the injection, the animals were killed, and samples of blood, ventricular muscle, lung, liver, pancreas, corpus striatum, and cerebellum were taken. The corpus striatum included the hippocampus along with a portion of the brain stem. No cerebral cortex or pineal gland tissue was sampled. Small samples of solid tissue (100–200 mg) and blood (25 μl) were processed overnight in tissue solubilizer and then counted after the addition of liquid scintillation cocktail. Replicate counts separated by 24 hr were taken to verify the absence of chemiluminescence. Samples containing ^{125}I were counted in a NaI scintillation counter. The

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TABLE 1
Stereoisomers of 4-IQNB

Compound	Designation	Quinuclidinyl center	Benzilic center	Reference
4-IQNB	R,R-IQNB	R	R	present study
4-IQNB	S,RS-IQNB	S	RS	present study
4-IQNB	R,RS-IQNB	R	RS	present study
4-IQNB	R-IQNB	R	R*	2
4-IQNB	R-IQNB	R	R*	5
N-M-4-IQNB	RS-IMQNB	RS	RS	6
4-IQNB	RS-IQNB	RS	RS†	3
4-IQNB	RS-IQNB	RS	RS	4
4-IQNB	RS-IQNB	RS	RS	8

* Benzilic center was resolved by selective precipitation; compound was identified incorrectly as R,S mixture in original reference. Full account of this will be published (*22*).

† R(Benzilic), R(Quinuclidinyl) was used for carotid injection study.

results are expressed as the percentage of the injected dose per gram of wet tissue with the standard deviation.

RESULTS

The distribution of [^{3}H]QNB has been published for sacrifice times up to 24 hr (*2–4*). The saturability of this binding has now been tested by postinjection (chase) of 25 or 250 nmol of R-QNB 30 min after the injection of radioactivity (Fig. 1). The radioactivity in the striatum is constant for 24 hr but the cerebellar activity decreases to 40% in the control. The heart maintains a near constant level of radioactivity followed by a decrease to 11% between 4 and 24 hr. In general, the displacement is dose dependent in that the 250-nmol dose displaced more radioactivity than the 25-nmol dose. Hydrogen-3 is displaced from the heart, the corpus striatum, and the cerebellum, all known to contain mAChR.

The distribution of R,R-4-[^{125}I]IQNB differs from R-[^{3}H]QNB in several ways (Table 2). More radioactivity is detected in the lung for R,R-4-[^{125}I]IQNB than for R-[^{3}H]QNB. However, this radioactivity is released rapidly. This increased lung concentration is in contrast to the lower uptake in the corpus striatum, the cerebellum, and the heart. The radioactivity in the heart and the cerebellum clears more rapidly when R,R-4-[^{125}I]IQNB is injected as compared to R-[^{3}H]QNB.

Further evidence that 4-[^{125}I]IQNB binds to a saturable receptor is given by displacement (chase) experiments (Figs. 2 and 3). If 25 or 250 nmol of R-QNB is injected 30 min after the injection of the radioiodinated compound, R,RS-[^{125}I]IQNB, a dose dependent displacement is observed.

Stereoselectivity of binding also has been tested by studying 4-[^{125}I]IQNB prepared using S-quinuclidinol (Fig. 4). For QNB itself, the S-isomer had 1% of the binding constant found for the R-isomer. The S-quinuclidinol isomer of 4-IQNB has a lower affinity constant as compared to R,R-4-[^{125}I]IQNB and this is reflected by a lower concentration of ^{125}I in the tissues that contain mAChR (heart, corpus striatum and cerebellum) with approximately the same concentration in lung and pancreas.

DISCUSSION

There is now a great deal of information indicating that both R-[^{3}H]QNB and R,R-[^{125}I]IQNB bind to the mAChR by a receptor-specific process (1 to 8). In this respect, the operational definition of a receptor (*9*) and, hence, the definition of a receptor binding radiotracer has been applied and as a result these two radiotracers are valid mAChR binding ligands. The displacement studies show that receptor binding occurs although the displacement is not quantitative. The chase experiments involve a complicated set of variables including the total receptor concentration, the dissociation rate, and transport of the displacing ligand (*10*). They do show that the lower concentration of radioactivity observed following a coinjection of radioactive and nonradioactive ligand (*4*) is not entirely due to altered blood flow or surface permeability changes. If the high concentration of receptor and slow dissociation rates measured in vitro are the appropriate values in vivo, then the chase experiments are consistent with a receptor binding mechanism. Similar observations can be made concerning the distribution and displacement of R,R-[^{125}I]IQNB. The radioactivity in the heart and the cerebellum clears more rapidly when R,R-4-[^{125}I]IQNB is injected as compared to R-[^{3}H]QNB. This is due to the discrimination of receptor subtypes by R,R-4-[^{125}I]IQNB as compared to

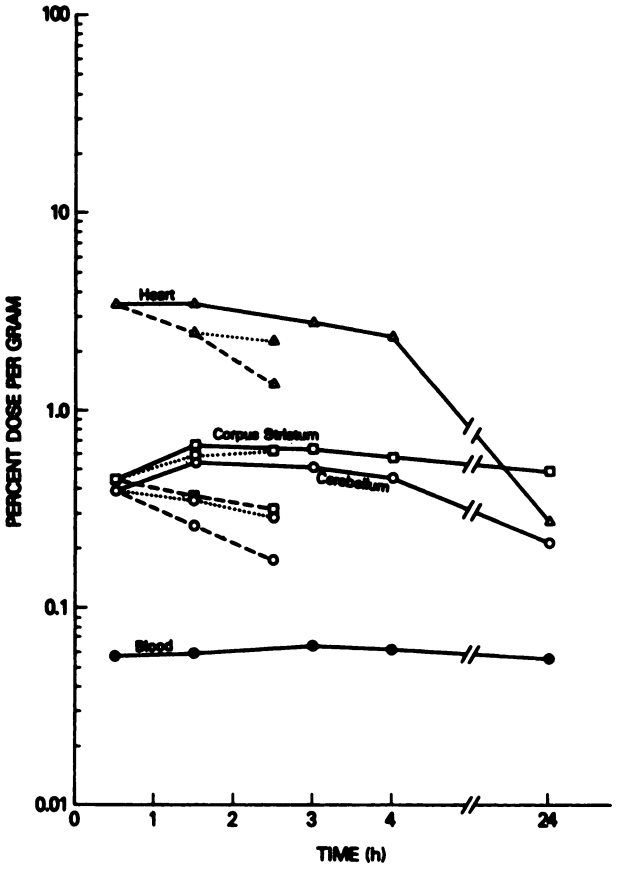

FIGURE 1
Displacement of R-[^{3}H]QNB from target organ by injection of either saline(–), 25(- - -) or 250(– –) nmol of R-QNB 30 min after injection of radioactive ligand

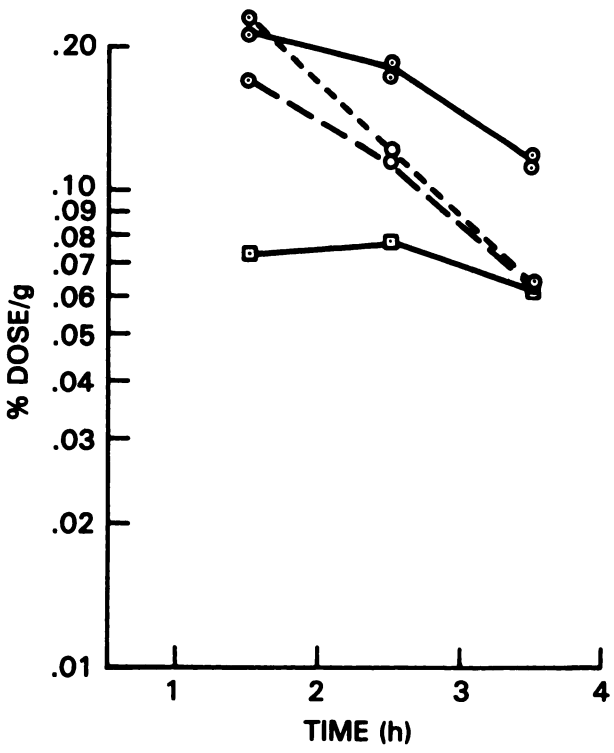

FIGURE 2
Displacement of R,RS-[^{125}I]QNB from heart by injection of either saline(–), 25(- - -) or 250(– –) nmol of R-QNB 30 min after injection of radioactive ligand. ⊙ Heart; (⊡) Blood

R-[^{3}H]QNB. R,R-4-[^{125}I]IQNB binds to the M_2 receptor in both heart and cerebellum with a lower equilibrium constant than to the M_1 receptor in the striatum mainly due to a faster dissociation rate, whereas R-[^{3}H]QNB binds to both receptor subtypes with the same affinity (4).

The distribution of S,RS-4-[^{125}I]IQNB compared to that for R,R-4-[^{125}I]IQNB shows that receptor binding is an important factor. The concentration of radioactivity in heart, corpus striatum, and cerebellum is lower and the rate of decrease is faster in the corpus striatum for the pharmacologically inactive isomer S,RS-4-[^{125}I]-IQNB. Other mechanisms of concentration such as pH shift (*11*) are not likely because the pKa for the amine in quinuclidinol should be the same for both stereoisomers.

The second criteria-measurement of change in receptor concentration—is difficult to observe for R-[^{3}H]QNB. The ratio of radioactivity in the corpus striatum (CS) and the cerebellum (CB) is 1.1, 1.2, 1.2, 1.3 at the time points up to 4 hr although the receptor concentration from in vitro saturation analysis indicates a ratio of 12 should be obtained (*12*). At 24 hr, the ratio of radioactivity rises to 2.3. Therefore, as a function of time, the ratio of radioactivity in the striatum and cerebellum increases but does not achieve the ratio found in vitro. This behavior has been predicted from simulations using similar binding constants (*13*). The changes in the CS/CB ratio for R,R 4-[^{125}I]IQNB cannot be attributed to the difference in concentration of receptors alone but also to different dissociation rates for binding of 4-[^{125}I]IQNB to the M_1 receptor (CS) and the M_2 receptor (CB) (5).

Previously [^{3}H]QNB distribution in rats has been shown to better reflect the in vitro-determined receptor concentration (*14*). [^{3}H]QNB of low specific activity was used (60 μCi of 4Ci/mmol compared to 10 μCi of 40 Ci/mmol in the present study). In the low specific activity study, at 1 hr the corpus striatum contained seven times the radioactivity found in the cerebellum. The uptake into the target tissue was rapid (5 min) and the cerebellum cleared in 50 min. It appears that the difference in receptor concentration is best reflected by the radioactivity in the tissue when low specific activity material is used. This concept is evident in the use of equilibrium binding studies such as radioimmunossay. The most sensitive part of the binding curve occurs near saturation; in fact the IC_{50} is usually at the midpoint of the linear portion of the binding curve relating percent bound and added ligand. However, the same phenomena occurs when the ligand concentration is held constant and the receptor concentration is varied (Fig. 5). With

TABLE 2
Distribution of R,R-4-[^{125}I]IQNB in Rat

Tissue	0.25	0.50	1.5	2	3	4	24
Blood	0.075 ± 0.031	0.061 ± 0.014	0.073 ± 0.057	0.051 ± 0.005	0.136 ± 0.233	0.126 ± 0.021	0.031 ± 0.013
Lung	5.66 ± 3.33	3.79 ± 1.52	3.66 ± 4.80	1.130 ± 0.245	0.722 ± 0.259	0.672 ± 0.388	0.034 ± 0.010
Heart	1.34 ± 0.684	0.970 ± 0.245	0.505 ± 0.113	0.320 ± 0.030	0.212 ± 0.108	0.120 ± 0.013	0.011 ± 0.004
Liver	0.826 ± 0.464	0.903 ± 0.278	0.503 ± 0.107	0.525 ± 0.057	0.446 ± 0.174	0.370 ± 0.096	0.197 ± 0.058
Pancreas	0.870 ± 0.460	0.923 ± 0.251	0.851 ± 0.416	0.582 ± 0.048	0.417 ± 0.251	0.244 ± 0.051	0.013 ± 0.004
Cerebellum	0.255 ± 0.154	0.209 ± 0.085	0.157 ± 0.142	0.121 ± 0.027	0.116 ± 0.100	0.048 ± 0.014	0.004 ± 0.002
Corpus striatum	0.299 ± 0.187	0.345 ± 0.143	0.302 ± 0.157	0.428 ± 0.060	0.422 ± 0.129	0.283 ± 0.045	0.153 ± 0.023

* All values are % dose/g wet tissue ± s. d. for >5 rats per value.

time, high specific activity R-[^{3}H]QNB approaches a maximum value of 2.3 at 24 hr but apparently is at too high a specific activity to reflect ratios equal to the receptor concentration, as measured in in vitro experiments. When there is a large difference between receptor concentration in the corpus striatum and cerebellum such as has been reported for man, the striatum-to-cerebellum ratio is high. In man, injection of R,R 4-[^{123}I]IQNB produced 5% of the dose in the caudate putamen with no detectable dose in the cerebellum (*2*). Larger differences in receptor concentration will produce larger changes even on the insensitive part of the curve. However, this study is complicated by the faster dissociation rates for 4-[^{123}I]IQNB in the cerebellum as compared to the caudate. The use of animal models with tissues containing small differences in receptor concentration is an important model to test the sensitivity of the system. The studies of Yamamura et al. (*14*) using low specific activity material are probably not predictive for the case of high specific activity material.

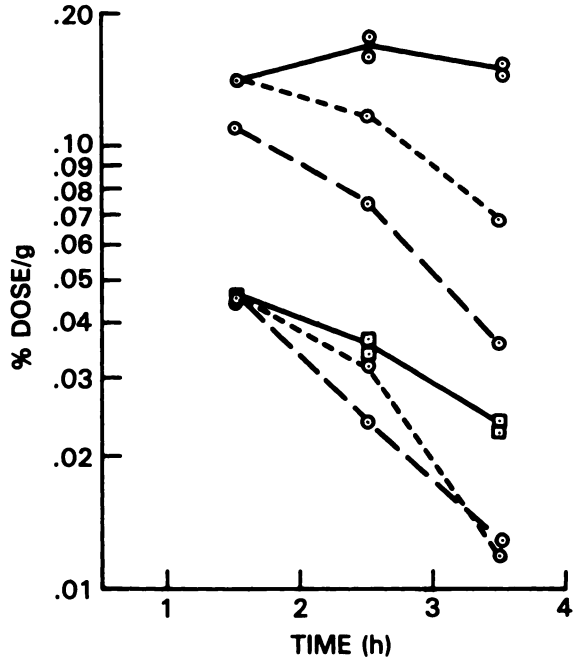

FIGURE 3
Displacement of R,RS-[^{125}I]IQNB from corpus striatum (⊙) and cerebellum (⊡) by injection of either saline 25(- - -) or 250(– –) nmol of R-QNB 30 min after injection of radioactive ligands

The sensitivity to changes in receptor concentration for R-[^{3}H]QNB are small compared to those reported by Wagner et al for a carbon-11- (^{11}C) labeled spiperone derivative. Wagner et al. (*15*) showed that the concentration of radioactivity in the caudate-putamen decreased as a function of age in man using [^{11}C] *N*-methyl spiperone (NMSP). They suggested that this decrease was related to a decrease in receptor concentration. They injected 10 μg/kg of 55 Ci/mmol [^{11}C]NMSP into mice and found the striatal activity to be 15 to 20 times greater than the cerebellar radioactivity at 1 hr. This amount of NMSP is not on the linear portion of the binding curve and for human studies the dose was decreased further to 1 μg/kg.

Possible explanations for this lack of difference between the concentration of ^{3}H in the striatum and the cerebellum in the rat using high-specific-activity R-[^{3}H]QNB and the measurable differences in the cand-

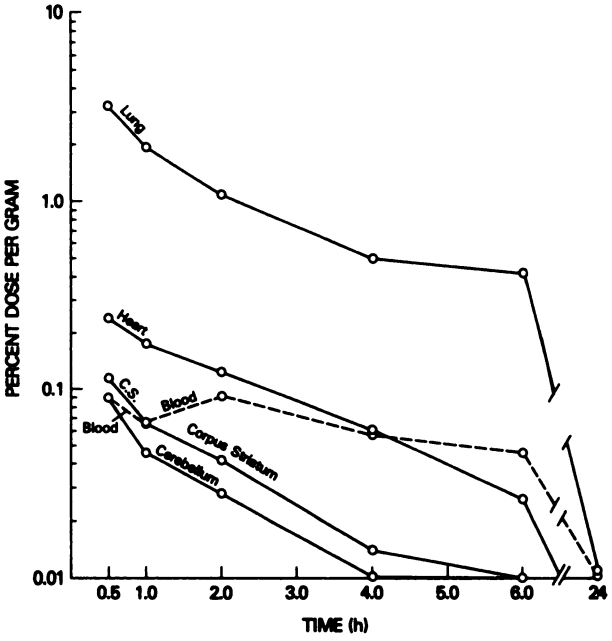

FIGURE 4
Distribution of S,RS-[^{125}I]IQNB in rat

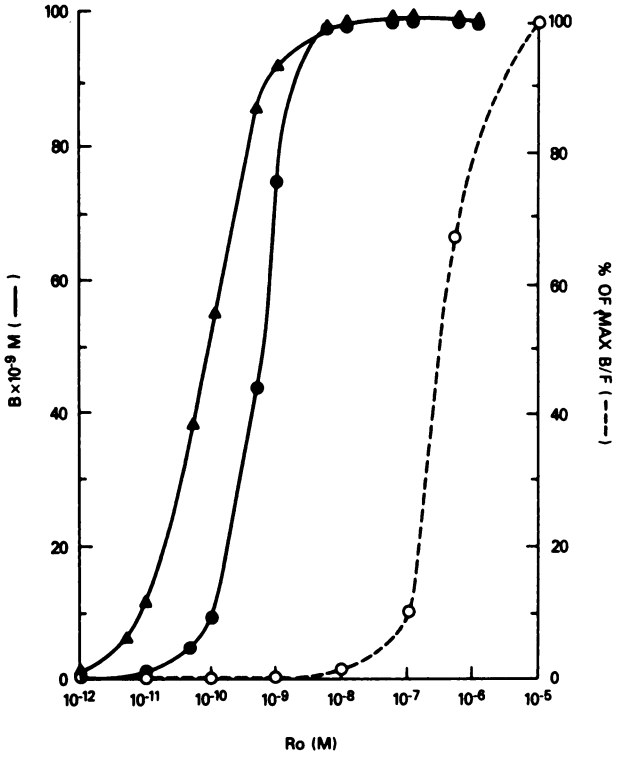

FIGURE 5
Relationship between concentration of bound radioligand binding and receptor concentration. B/F ratio(- - -) is also given. (●) L = 10^{-9}; (▲) L = 10^{-12}; (K) 12.9 X 10^{9} M^{-1}

ate-putamen of aging man using [^{11}C]NMSP are many. Such problems as partial volume effects (*16*) and flow changes with age (*17*) could contribute to the decrease but seem unlikely (*18*). The differences in dopamine receptor concentration in aging man may be much larger than the factor of 10 difference in rat mAChR and thus would give a large ratio of radioactivity. However, Seeman showed that the change in dopamine receptors in schizophrenic patients was increased by a factor of only two (*19*) and Wagner et al. (*15*) report that various studies in animals showed no more than a factor of 2 decrease in receptor concentration. The difference in specific activity for the Yamamura et al. (*14*) study and the present work is sizable; approximately 170 times more mass was used in the Yamamura et al. study (28 μg QNB/kg). The dopamine receptor study of Wagner et al. (*20*) used an intermediate amount of material but the receptor system is different. Depending on differences in metabolism and protein binding, Wagner et al. may be near to the linear portion of the curve, and this would explain the higher sensitivity to receptor concentration change. Certainly, NMSP has the highest specific concentration in the brain of those dopamine antagonists tested (*21*).

In conclusion, more extensive animal distribution studies using radiotracers with various specific activities and assaying tissue with small differences in receptor concentration are needed. Only by this approach can the sensitivity of receptor binding radiotracers be determined. Iodinated QNB has been shown to bind to mAChR in vivo; but further studies are needed to demonstrate the sensitivity of uptake in target organs to a change in receptor concentration.

FOOTNOTE

* DuPont NEN Medical Products, No. Billerica, MA.

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