RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

The Characteristics of I-125 4-IQNB and H-3 QNB In Vivo and In Vitro

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The accumulation of (R)-(H-3)-3-quinuclidinyl benzilate (H-3 QNB) and (R,S)-1-azabicyclo(2.2.2)oct-3-yl (R,S)-alpha-hydroxy-(4-[I-125]iodophenyl)benzeneacetate (I-125 4-IQNB) in heart, caudate/putamen, and cerebellum of rats was determined at intervals from 15 min to 4 hr after injection. The behavior of the two radiotracers in the heart is consistent with in vitro results with respect to affinities and specificities. In the brain, however, the compounds differ in tissue selectivity. At high specific activity, neither compound provides localization that is consistent with the concentration of receptor in the tissues. The results of this study do not indicate quantification of receptor concentration by means of single external images.

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The muscarinic acetylcholine receptor (m-AChR) is present in the heart (*1–3*), pancreas (*4–6*), intestinal smooth muscle (*7,8*), and many structures of the brain (*9–11*). We have suggested that a suitably labeled radioligand (either I-123 or F-18) specific for the m-AChR may be useful for the study of changes in receptor concentration as a function of disease (*12*). To this end we have prepared (R,S)-1-azabicyclo(2.2.2)oct-3-yl(R,S)-alpha-hydroxy-alpha-(4-[I-125]iodophenyl)-benzeneacetate (I-125 4-IQNB, Fig. 1) and studied its behavior in vivo in the heart and in two structures of the brain, the caudate/putamen (CP) and the cerebellum (CB), with the latter exhibiting one-tenth the receptor concentration of the former. The affinity of the 3- and 4-fluorobenzilate analogs of QNB are essentially the same as that of QNB (*13*). We therefore use commercially available (R)-3-quinuclidinyl [^{3}H] benzilate (H-3 QNB) in place of the fluoro-analogs.

MATERIALS AND METHODS

The iodinated QNB analog (I-125 4-IQNB) was synthesized by the reaction of iodide with the 4-triazeno derivative of QNB, in a modification of the procedure of Tewson et al. (*14*), with an overall yield of 20%. The Wallach reaction (*15*) with polyfunctional substrates is not trivial; therefore, the details of the synthetic procedures and complications are described elsewhere (*16*). The product, isolated by reversed-phase HPLC, had an elution volume identical with that of an authentic sample of stable tracer 4-IQNB (analysis within 0.4% of theoretical). There is no UV peak coincident with the peak of radioactivity; nonetheless, the specific activity of the I-125 4-IQNB used in these studies varied from 900 to 1200 Ci/mmol. Trailing of UV absorbing peaks that elute before the desired product is the most probable cause of the reduced specific activity. Radiochemical purity was determined by TLC (95%) and the product also 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). (R)-[H-3]QNB was purchased,* (33.1 Ci/mmol). (R,S)-QNB and (R,S)-QNX (Fig. 1) were synthesized in our laboratory (*13*).

Distribution studies. *Venous injection.* Five microcuries of radioligand (labeled with either H-3 or I-125), in 0.1 ml normal saline containing 20% EtOH, was injected through either tail vein or femoral vein into female Sprague-Dawley rats (av. wt. 250 g). At various times (from 5 min to 4 hr) the animals were killed by cervical

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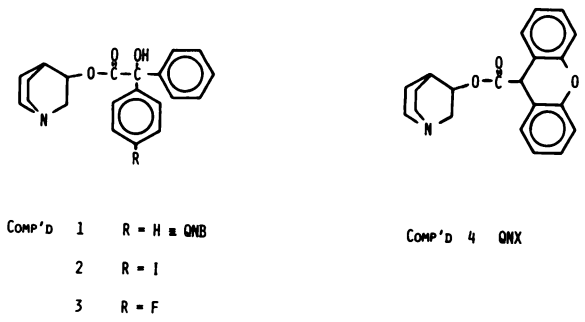

FIG. 1. Iodine-125 4-IQNB.

dislocation under mild ether anesthesia. The hearts and brains were immediately removed and placed on ice. In the case of H-3, 10- to 20-mg samples of tissue were excised, blotted free of excess blood and solubilized in 1 ml of Protosol*. After neutralizing excess base with acetic acid, 15 ml of Econofluor were added, and the samples were dark-adapted and counted for 5 min each in a scintillation counter. Lack of bioluminescence was determined by repeated counting of the samples. Corrections to dpm were made using the counter's external standard and a quench curve. For I-125, the samples were counted in a gamma counter for 5 min. In all studies, a minimum of five animals was used per reported result unless otherwise stated.

Carotid artery injection. Ten microcuries each of H-3 QNB and I-125 4-IQNB were injected as described above in the exposed right common carotid artery of female Sprague-Dawley rats (200–250 g) under ketamine analgesia. The volume of injectate was 0.1 ml (30% EtOH in normal saline) and injection was accomplished in less than 2 sec. At either 5 or 15 min, each animal was killed by cervical dislocation and samples from the heart, brain, lung, liver, and blood were immediately placed on ice. The brains were divided into right and left halves and the caudate nucleus and cerebellum removed. After preparation, the samples were first counted on a gamma scintillation counter. The samples were then counted for both I-125 and H-3 on a liquid-scintillation counter capable of discriminating I-125 and H-3. We find that our instrument provides good estimates of activity for both nuclides when the ratio of activities is less than 10:1 and each is $<1.35\ \mu$Ci (50 KBq). The results for the crystal and the liquid scintillation counters agree to within 95% confidence limits. The results are the average of five animals per time interval.

Filtration studies. The tissue preparation for heart and the procedure for the determination of Kapp values has been described previously (*13*). The reported Kapp values are the result of at least five determinations as previously described (*13*), calculated using the ligand program of Munson and Rodbard (*17*). The 95% confidence intervals are calculated by the method of Munson and Rodbard (*18*).

Off-rate determination. The concentration of m-AChR in the CP is ten times that in the (CB). We therefore prepared the tissues differently. For the CP, 0.1 to 0.15 g of tissue was homogenized in 10 ml of 10 m*M* Tris-buffered saline (pH 7.4) containing 8% sucrose. The crude homogenate was used without further preparation. Four to 5 g of CB were homogenized in 7 volumes of the above buffer. After centrifugation at 2000 *g* for 10 min, the supernatant is recentrifuged at 100,000 *g* for 1 hr. The P2 pellets thus obtained are suspended in 10 ml of the initial buffer for assay. Receptor recovery is 20%.

The receptor from CP and CB at 2–5 n*M* was incubated with 20 p*M* I-125 4-IQNB for 2 hr to achieve maximum receptor binding. Afterward, 0.1-ml samples were diluted into 5 ml of Tris-buffered saline containing 10-μM atropine and the mixture filtered at times from 10 sec to 4 hr, then washed with 9 ml of ice-cold saline. Values for t_∞ were determined by incubating aliquots of the receptor-radioligand preparations with 10-μM atropine for the time of the initial incubation. The temperature was maintained at 30°C for the off-rate determinations.

Specific activity determination. The specific activities of the I-125 4-IQNB preparations were determined by matching receptor concentrations to that determined using H-3 QNB as a standard, as previously described (19).

RESULTS AND DISCUSSION

The accumulation of H-3 QNB at 2 Ci/mmol in heart, CP, and CB, following intravenous injection, is presented in Fig. 2A. Peak levels are obtained by 15 min. Although absolute levels obtained in the brain seem highest at 2 hr, there is no statistically significant difference between the value obtained at 15 min relative to later times. In the heart a gradual washout of activity is seen, with 50% loss of activity by 4 hr. By contrast, the activities in the CP and CB do not significantly change over 4 hr. The activity that localizes in these tissues is the result of the interaction with the m-AChR, since coinjection of (R)-QNB (50 nmol/rat for heart and 500 nmol/rat for CP and CB) results in 95% blockade of activity in the heart, CP, and CER (*20*).

The accumulations of I-125 4-IQNB at 1077 Ci/mmol in the heart, CP, and CB are presented in Fig. 2B. In the heart, peak levels of activity are achieved within 15 min. The washout of activity is faster than that with H-3 QNB (60% at 2 hr and 85% by 4 hr). The peak of activity (0.4% dose/g) is 1/6 of that obtained by H-3 QNB. The activity in the CP and CB reaches peak levels by 30 min. As with H-3 QNB, there is no washout of activity from the CP. There is, however, significant washout from the CER (90% by 4 hr), which leads to a CP-to-CB ratio of 7 at 4 hr. In addition, there is no

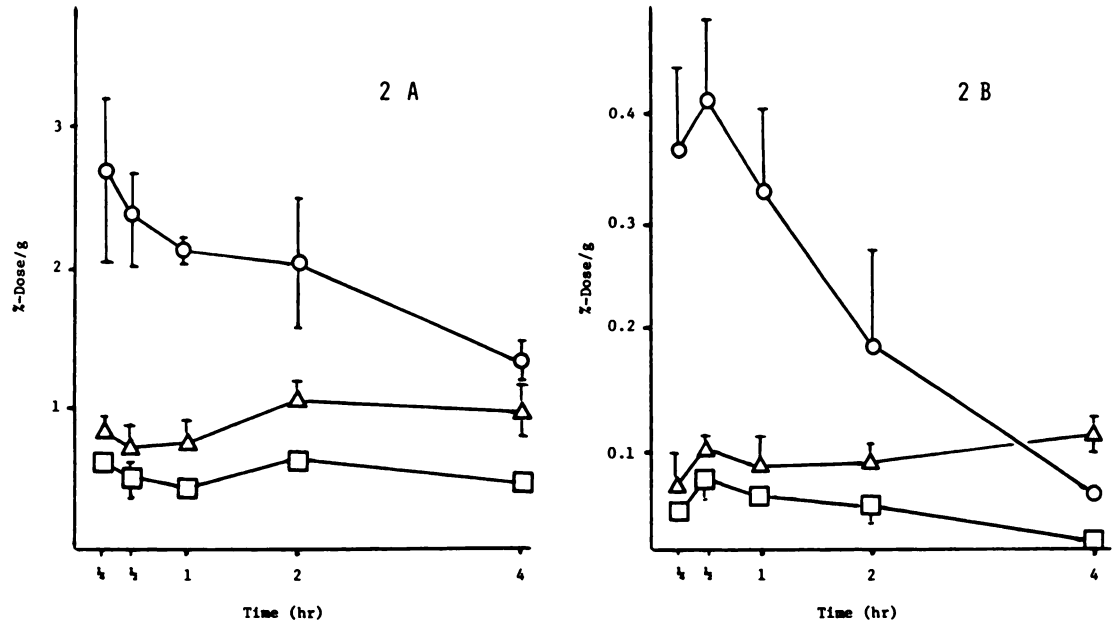

FIG. 2. A. Distribution of H-3 quinuclidinyl benzilate (2 Ci/mmol) in heart (O), caudate/putamen (△) and cerebellum (□) at various times after injection. B. Distribution of I-125 4-IQNB (1077 Ci/mmol) at various times after injection. Note that y-axis has different scale.

change in that ratio when the specific activity is diluted from 1000 Ci/mmol to 2 Ci/mmol with stable 4-IQNB. The peak levels of activity in the CP and CB are $^1/_{10}$ of that observed with H-3 QNB. However, these levels are sufficient to provide planar images of dog brain and single photon emission computerized tomography (SPECT) in man (unpublished results). Another compound, (R,S)-1-azabicyclo(2.2.2)oct-3-yl (R,S)-alpha - hydroxy - alpha-(3-hydroxy-4-[^{123}I]iodophenyl)-benzeneacetate I-123 HO-IQNB, has also been used to image the calf brain (*21*). We find that I-125 HO-IQNB has a lower affinity and higher nonreceptor binding than I-125 4-IQNB.

As in the case of H-3 QNB, co-injection of (R,S)-QNB blocks the accumulation of I-125 in the target tissues. But only 50% of the I-125 4-IQNB that localizes in the heart is blocked by 50 nmol of (R,S)-QNB. Co-injection of 500 nmol of (R,S)-QNB blocks 80% of the I-125 4-IQNB that localizes in the CP and CER (*20*). These results are in agreement with those reported by Drayer et al. (*21*): injection of scopolamine, another potent m-AChR antagonist, reduced the concentration of I-123 HO-IQNB in the calf brain as determined by both SPECT and postmortem samples.

Although H-3 QNB and I-125 4-IQNB are rapidly cleared from the blood, thin-layer chromatography of target tissue samples indicates that the activity in those tissues has not undergone metabolic transformation.

TABLE 1A. COMPARISON OF H-3 QNB AND I-125 4-IQNB DISTRIBUTION FOLLOWING RIGHT CAROTID ARTERY INJECTION*

Organ	Radiotracer	%-dose/g	
		5 min	15 min
Right caudate	H-3	6.62 (4.72–8.85)†	6.45 (4.33–8.56)
	I-125	6.53 (4.63–8.46)	5.22 (3.42–8.00)
Left caudate	H-3	0.272 (0.230–0.310)	0.400 (0.290–0.550)
	I-125	0.201 (0.168–0.233)	0.383 (0.205–0.561)
Right cerebellum	H-3	0.581 (0.170–0.990)	1.27 (0.389–2.15)
	I-125	0.360 (0.106–0.614)	0.822 (0.204–1.44)
Left cerebellum	H-3	0.325 (0.120–0.530)	0.278 (0.190–0.479)
	I-125	0.225 (0.088–0.361)	0.237 (0.144–0.331)
Whole brain	I-125	2.83 (2.18–3.49)	R 5.30 (2.25–8.34)
			L 0.457 (0.137–0.778)

* 10 μCi of each radiotracer coinjected as a rapid bolus.
† 95% confidence intervals in parentheses.

TABLE 1B. COMPARISON OF H-3 QNB AND I-125 4-IQNB SYSTEMIC DISTRIBUTION FOLLOWING CAROTID ARTERY INJECTION

Organ	Radiotracer	%-dose/g		
		5 min	15 min	15 min*
Heart	H-3	2.87 (2.20–3.54)	3.94 (3.42–4.47)	3.78†
	I-125	2.20 (1.85–2.56)	2.25 (1.61–2.89)	1.03
Lung	H-3	3.75 (2.49–5.02)	2.57 (0.722–4.41)	2.50
	I-125	9.43 (7.21–11.6)	7.70 (6.02–9.40)	5.57
Liver	H-3	0.934 (0.710–1.16)	0.631 (0.524–0.738)	
	I-125	1.80 (1.15–2.45)	1.97 (1.15–2.78)	
Plasma	H-3	0.154 (0.086–0.220)	0.178 (0.146–0.198)	0.20
	I-125	0.090 (0.046–0.134)	0.104 (0.093–0.114)	0.12

* Distribution of activity following intravenous injection.

† Data on the I-125 4-IQNB distribution from Ref. *20*. Data on H-3 QNB distribution are from Ref. *3*.

Activity in the liver, however, did not migrate from the origin in either solvent system described under Methods.

The lower concentrations of I-125 compared with H-3 observed at 15 min in both the CP and CB may result from either lower permeability of the iodinated compound or from reduction of the concentration of I-125 4-IQNB in the inital intravenous bolus by sites of loss, such as lung (lipophilic localization) and liver (metabolic clearance). Carotid artery injection will, for the first pass, eliminate systemic losses of I-125 4-IQNB compared with H-3 QNB. The results in Table 1A clearly show that the levels of activity in the CP for the two radiotracers are comparable at 5 min and 15 min following carotid injection. The %-dose/g for the CB is considerably lower, however, than that in the CP. Injection into the common carotid does not deliver a first-pass dose to the CB. Comparison of activities in the CP and CB from the left hemisphere, where systemic losses of activity are expected, are consistent with the i.v. injection. The right half of the CB exhibits higher levels of activity than the left, but the differences are not significant.

In addition to the activity in the brain, we examined the distribution of activity in other organs of interest at 5 and 15 min (Table 1B). Activity in the lung is much greater than in the heart, whereas in the liver it is slightly less. We have included data obtained 15 min after i.v. administration of the radiotracers for comparison, and it is apparent that our previous results are in agreement. Note that the I-125 4-IQNB used for the carotid artery injection was prepared from resolved (R)-3-quinuclidinol, which may lead to a twofold difference in the %-dose/g obtained in target organs such as the heart. The results in Table 1 are consistent with the interpretation that the lower levels of I-125 4-IQNB that reach the target tissues, as compared with the levels observed for H-3 QNB, result from greater systemic losses, namely, increased trapping in lung and increased clearance by the liver.

TABLE 2. CAUDATE/PUTAMEN TO CEREBELLUM RATIO AT 2 hr FOR H-3 QNB*

Specific activity (Ci/mmol)	Ratio
33	1.2
8	1.4
2	1.7
1	2.8
0.1	3.2
0.01	1.2

* Data from Ref. *20*.

At 33.1 Ci/mmol, the CP-to-CB ratio for H-3 QNB—supposedly representing ratio between abundant compared with scanty receptors in the target tissues—is only 1.2. This is in contrast to the ratio of 3.6 reported by Yamamura et al. (*23*). They injected 60 μCi of H-3 QNB at 4 Ci/mmol, 100 times the mass used in our study. When we reduced the specific activity to 0.1 Ci/mmol (3 times the mass used by Yamamura et al.), the CP-to-CB ratio was increased to 3.2 (Table 2). A further 1:10 reduction in specific activity reduced the ratio to 1.2. This effect on the CP-to-CB ratio cannot be understood easily in either biochemical or physiological terms. For example, we have determined that the affinity of H-3 QNB for the m-AChR in the CP and CB is the same; and cerebral blood flow to the CP and CB in the rat differs by only 10% (*23*). However, when the concentration of receptor-binding radiotracer is much less than that of its specific receptor, the observed effect of specific activity can be explained by simple competition for the m-AChR by an unlabeled ligand. Since the concentration of H-3 QNB is much less than that of its receptor, we cannot use the simple equation for inhibition of radiotracer binding in which the ligands are assumed to be in excess of the receptor concentration:

$$B = \frac{K_A R_0 L_0}{1 + K_A L_0} \quad (1)$$

[For the definition of the symbols, see the discussion of Eq. (2)].

The interaction of radiolabeled and unlabeled ligands with receptors (under any condition of receptor or ligand concentration) can be described theoretically by a cubic equation (*24*), which provides an explanation for the change in the CP-to-CB ratio as a function of the specific activity, and requires only that the concentration of the receptor in those two structures be different. The binding of a radiotracer to a receptor in the presence of an inhibitor can be explained by the following equation:

$$K_A B^2 + B(-1 - K_A L_0 - K_A R_0) + K_A L_0 R_0 + \frac{I_0 K_h K_A B(B - L_0)}{K_h B + K_A(L_0 - B)} = 0, \quad (2)$$

where K_A and K_h are, respectively, the affinity constants of the radioligand (L_0 = initial concentration) and the competing ligand (I_0 = initial concentration) for the receptor, which has an initial concentration of R_0; B is the molar concentration of bound radiotracer. The values of K_A and K_h are obtained from in vitro equilibrium radioligand/receptor saturation studies. Since the radioligand and the competing ligand are chemically alike, $K_A = K_h$. The concentration of receptor in the tissues has also been determined in vitro. R_0 for the CP is approximately 50 to 100 pmol/g of wet tissue. If we assume uniform distribution of receptors, the concentration of receptor would be 10^{-7} *M*. The concentration of receptor in the CB is $^1/_{10}$ that of the CP, or $\sim 10^{-8}$ *M*. Using these values for affinity constant and receptor concentration, the cubic equation provides a theoretical framework by which we can examine the effect of reducing the specific activity on the amount of radiotracer bound in the CP and CER. From the %-dose/g at 5 min after injection, and assuming uniform distribution of activity, we can estimate that the ligand concentration (L_0) in the CP and CER is 150 p*M*. From the experimental data we know that at least 5% of the activity present in the CP and CB is not associated with the m-AChR. Using these initial parameters in Eq. (2), we have calculated the theoretical CP-to-CER ratios for six concentrations of added (R)-QNB (Table 3). We used (R,S)-QNB in the animal studies, but the affinity of (S)-QNB for the m-AChR is 1% of the affinity of (R)-QNB, so we assume that only the (R)-QNB is active. From Eq. (2) the ratio of ligand bound in the CP to the ligand bound in the CB is 1.1 when $I_0 = 0$. As the value of I_0 is increased—equivalent to reducing the specific activity in the experimental situation—the ratio increases. This paradoxical behavior occurs because the concentration of competitor begins to saturate the m-AChR in the CB even though its concentration is still far less than that of the receptor in the CP. Thus the unlabeled ligand selectively blocks radiotracer binding in the structure that has the lower concentration of m-AChR. At higher concentrations of I_0, the specific binding of radiotracer to CP is also competitively blocked.

TABLE 3. THEORETICAL CAUDATE/PUTAMEN TO CEREBELLUM RATIO AS A FUNCTION OF ADDED UNLABELED (R,S)-QNB (I_0)

I_0	CP/CER
10 n*M*	1.11
30 n*M*	2.76
100 n*M*	6.80
300 n*M*	4.60
1000 n*M*	2.50
10,000 n*M*	1.35

Several points should be noted concerning these calculations. First, although a theoretical maximum of 10 is possible, the addition of 5% nonreceptor localization of radiotracer reduces the theoretical value to 6.8. Second, the theoretical calculations indicate that, in the absence of nonreceptor binding, a ratio of 10 is maintained to very low specific activities, but the experimentally observed ratio must approach 1, since activity associated with nonreceptors (the 5% nonreceptor level) must become dominant as the specific binding is reduced to 0%. Third, although we have observed ratios no greater than 3.2, we have added I_0 in 1:10 increments, large enough to have missed the maximum ratio.

The above equation is based upon two assumptions: (a) that the equilibrium equation can be used to describe the binding of these radiotracers in the brain, and (b) that the distributions of L_0 and R_0 are uniform. With respect to the first assumption, when the sum of the microscopic on-rate and off-rate is much larger than the loss of activity from the tissue, the ligand-receptor interactions can be treated using the equilibrium equation. This is analogous to the situation described by Notari (*25*) for the slower beta phase of drug clearance from plasma. Although we do not know the clearance rate from the extracellular fluid of the brain, our results suggest that the elimination constant from that compartment is at least as slow as the clearance from plasma. The on-rate for the binding of radioligand to receptor [$k(on) = 4 \times 10^8/\text{min}/n$] is sufficiently large, however, to suggest an alternate explanation: that the accumulation of activity would be flow-limited. If the extraction efficiency is high, this would also lead to the same levels of activity in the CP and CB (*26*). In addition, the effect of reducing the specific activity provides CP-to-CB ratios that do not differ significantly from those in Table 2, and thus would not provide a means of discriminating between the two methods of describing the data. We do not have data on the permeability of the blood-brain barrier for either I-125 4-IQNB or H-3 QNB. Preliminary

studies on the extraction efficiency of I-123-labeled 4-IQNB in the monkey indicate, however, that the binding of the radiotracer is not entirely flow-limited: extraction efficiency of 54% at normal flow and 37% at 100 ml/min·100 g (unpublished results in collaboration with Dr. M. J. Welch). We therefore suggest that the blood-brain barrier reduces the extraction efficiency enough so that flow is not the dominant factor in the accumulation of activity in the CP and CB. Therefore the equilibrium model is most consistent with the data.

It is also clear that neither the radioligand (L_0) nor the receptor (R_0) is uniformly distributed. With respect to the assumption of uniform distribution of L_0, the extent of nonreceptor binding has been estimated as 5% of the total ligand present in the tissue. Although this was determined by coinjection of a large excess of unlabeled QNB, we think the in vitro assay results indicate that this extent of nonreceptor binding is reasonable for H-3 QNB. As such, the contribution of nonreceptor binding is small and does not become significant until the proportion of receptor-specific binding is reduced severely. The receptor is clearly not uniformly distributed, but is localized in synapses within the structures of interest. There is no evidence to suggest that the receptor levels in the synapses of the CP and CB are comparable. However, if the receptor density within the synapses is equivalent, the number of synapses within the CB must be at most $^1/_{10}$ of that in the CP. The net effect of the reduced density is apparently the same as if the receptor were more dilute. Because of these assumptions, we do not consider it appropriate at this time to attempt a quantitative description of the accumulation and washout of activity from the two structures of interest, and have presented the results in Table 2 qualitatively as ratios. Within the constraints of these rather extensive caveats, the cubic equation provides a good description of the relative binding of a radiotracer and its selective competitive blockade by an unlabeled ligand (i.e., dilution of the specific activity) when the only difference between the m-AChR in the two structures with respect to H-3 QNB is the concentration of receptors!

The results obtained for H-3 QNB and I-125 4-IQNB in heart are consistent with in vitro results. First, the ratio of receptor to nonreceptor binding in vitro, as assayed by filtration on GF/C filters, is at least 10:1 for H-3 QNB but only 1:1 for I-125 QNB. This large degree of nonreceptor binding is attributed to interactions with contractile proteins; similar interactions have been demonstrated for lipophilic beta-adrenergic receptor antagonists (*27*). Second, the faster washout of I-125 QNB relative to H-3 QNB is consistent with the one-fourth affinity exhibited by 4-IQNB for the m-AChR in the heart (Table 4).

Two differences are seen between the behavior of I-125 4-IQNB and H-3 QNB in the brain. First, I-125 4-IQNB does not obtain the same levels of activity in the brain as H-3 QNB following i.v. injection. Since the levels of H-3 and I-125 are the same following arterial injection, I-125 QNB exhibits greater systemic losses than are seen with H-3 QNB. Therefore the concentration of ligand obtained in the brain after i.v. injection is $^1/_{10}$ of that obtained with H-3 QNB. When the specific activity of I-125 QNB was diluted to 2 Ci/mmol, there was no change in the CP-to-CB ratio. Because of the 1:10 reduction of ligand concentration that reaches the brain (due to systemic losses), the mass of I-125 4-IQNB in the brain is equivalent to 20 Ci/mmol for H-3 QNB—a level that would not produce ratios much greater than 1 (see Table 1). Second, and more importantly, I-125 4-IQNB exhibits considerable washout from the CB but not from the CP. We have recently reported (*28*) that the m-AChR may exist in at least two subclasses, m_1 and m_2. The affinities of several compounds are provided in Table 4, where we have defined the m-AChR from heart as m_1 and that from the CP as m_2. Although the difference in affinity for m_1 and m_2 receptors is only twice that for

TABLE 4. EQUILIBRIUM ASSOCIATION CONSTANTS FOR m-AChR ANTAGONISTS

Compound No.*	K_{app} ($\times 10^{-9}$ M^{-1}) Heart†	CP
1	5.28 (3.7–7.5)‡	3.62 (2.8–4.7)
2	1.22 (.87–1.7)	2.37 (1.6–3.5)
3	3.03 (2.3–3.9)	3.97 (2.5–6.3)
4	0.225 (0.17–0.30)	3.56 (2.6–4.9)

* For structure, see Fig. 1.
† Data from Ref. *13*.
‡ 95% Confidence limits.

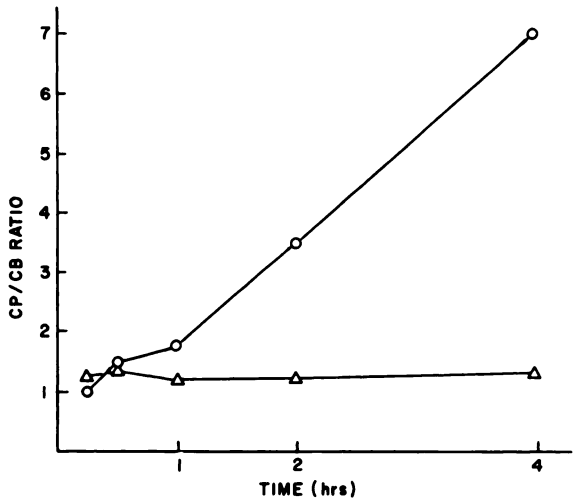

FIG. 3. Ratio of activity in caudate/putamen to cerebellum as a function of time: (O) I-125 4-IQNB, specific activity of 2 Ci/mmol or 1077 Ci/mmol; (Δ) H-3 quinuclidinyl benzilate, specific activity of 33.1 Ci/mmol.

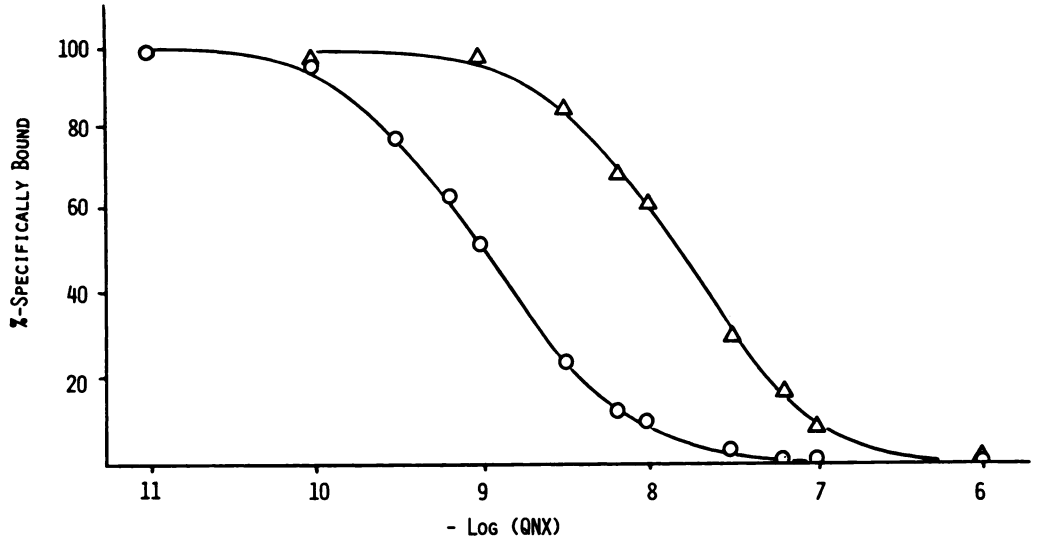

FIG. 4. Competition inhibition of H-3 quinuclidinyl benzilate to caudate/putamen (O) and cerebellum (Δ) by QNX (see Compound 4, Fig. 1). Affinities differ by 16:1, showing that the m-AChR subtypes in the CP and CB are different.

4-IQNB (Compound 2), we have reported a compound, QNX (Compound 4), that exhibits an affinity for the m-AChR in CP 16 times that found in the heart (Table 4). In order to define the subclass of receptor present in the rat CB, we performed competition studies using QNX. The affinity of QNX for the m-AChR in CB is $^1/_{10}$ of that obtained using CP preparations (Fig. 3). This suggests that the receptor in the CB is different from that found in the CP. Differences in the affinities of m-AChR antagonists for the receptors from different tissues and different structures of the brain have been reported (*8*,*28*,*29*).

In order to confirm these observations with the ligands of interest, we determined the off-rate of I-125 4-IQNB and H-3 QNB for receptor obtained from the CP and CB. The I-125 4-IQNB used in these studies was prepared using resolved (R)-quinuclidinol; i.e., (R)-1-azabicyclo(2.2.2)oct-3-yl (R,S)-alpha-hydroxy-alpha-(4-iodophenyl)benzeneacetate. The product nonetheless is a mix of stereoisomers around the carbinol chiral center. The results are presented in Fig. 4. Firstly, there is no difference in the off-rate of H-3 QNB for the receptor from CP and CB, within experimental error (k_{-1} = 0.012/min). Additionally, the off-rate of I-125 4-IQNB from the CP is similar to that of H-3 QNB (k_{-1} = 0.008/min).

The off-rate profile of I-125 4-IQNB from the CB is, however, at least biphasic. The curve can be resolved into two components with constants of 0.10/min (75%) and 0.008/min (25%). Since there is an unequal proportion of the two components, we think that these in vitro results cannot be explained by differences in the affinities of the

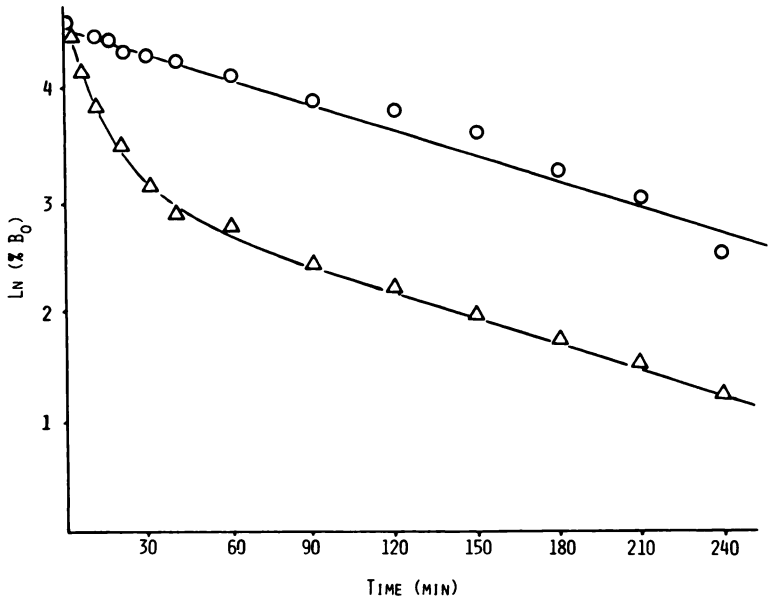

FIG. 5. Off-rate profiles of I-125 4-IQNB bound to caudate/putamen (O) and cerebellum (Δ). For the CP, k_{-1} = 0.0086/min. For CB, $k_{-1(fast)}$ = 0.10/min, and $k_{-1(slow)}$ = 0.008/min. Fast off-rate component in CB represents 76% of total bound radiotracer.

two stereoisomers. An off-rate of 0.1/min is enough to cause the increased washout of I-125 4-IQNB in vivo, but if rat CB contains 25% of the high-affinity receptor (m_2), we would not expect the 90% washout of the I-125 activity from the CB that is observed. It is likely that the preparation of the P2 pellet (20% recovery of receptor) for these studies may have enriched the proportion of m_2 receptor. In three studies the proportions of m_1 to m_2 receptor are 54:46, 70:30, and 76:24. These results have been found using rat and rabbit tissues.

CONCLUSION

The ratio of activity of H-3 QNB in the CP and CER does not provide an estimation of receptor concentration in those tissues when the specific activity is greater than 1 Ci/mmole. Low specific activities that would select for the target tissue with higher m-AChR levels will exhibit prohibitive CNS toxicity. We suggest that F-18 FQNB, which should exhibit properties similar to that of H-3 QNB (Table 3, Compound. 3), will not provide static images of brain structures concordant with the distribution of receptors in the brain. It follows that changes in receptor concentration as the result of disease will not be observed using high-specific-activity m-AChR antagonists with a single image. However, it has been suggested (*30,31*) that the kinetics of radiotracer washout from these tissues will provide the desired quantitation. Since H-3 QNB exhibits a very slow washout from the brain, a compound with lower affinity (perhaps on the order of that exhibited by I-125 4-IQNB for the heart m-AChR) may prove more useful for quantitating m-AChR in the brain than compounds with affinities as high as that of QNB.

FOOTNOTE

* NEN.

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Residency Listing

A list of all AMA approved residency programs in nuclear medicine appeared in the January issue of *JNM* on pages 33A and 34A.

The listing also appears in this issue, for your convenience, on pages 29A and 30A.

On both lists an "X" indicates those programs that still have openings available for 1984.