In Vitro and In Vivo Characterization of 4-[^{125}I]Iododexetimide Binding to Muscarinic Cholinergic Receptors in the Rat Heart

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4-[^{125}I]iododexetimide binding to muscarinic cholinergic receptors (mAChR) was evaluated in the rat heart. 4-[^{125}I] iododexetimide displayed high in vitro affinity (Kd = 14.0 n*M*) for rat myocardial mAChR. In vivo, there was high accumulation of 4-[^{125}I]iododexetimide in the rat atrium and ventricle which could be blocked by ~60% by preinjection of atropine. In contrast, accumulation of the radiolabeled stereoisomer, 4-[^{125}I]iodolevetimide, was 63% lower than 4-[^{125}I]iododexetimide and was not blocked by atropine. The blood clearance of 4-[^{125}I]iododexetimide was rapid, providing heart-to-blood ratios of up to 14:1; however, heart-to-lung and heart-to-liver ratios were below unity. The data indicate that 4-[^{125}I]iododexetimide binds potently to rat mAChR. However, since nonspecific binding is relatively high, it is not clear whether iododexetimide labeled with ^{123}I will be useful in SPECT imaging studies of myocardial mAChR. Further studies in humans are indicated.

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Muscarinic cholinergic receptors (mAChR) of the heart are important for cardiac function and have been reported to be altered in various conditions and diseases, such as aging (*1*), diabetes (*2*), and congestive heart disease (*3*). The widely used anti-arrhythmic agents, quinidine, lidocaine and procainamide, exhibit interactions with cardiac mAChR (4). The localization and quantitation of mAChR in the living human heart using a noninvasive method such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) may provide valuable information about receptor changes in various disease states and may be useful in monitoring drug interventions.

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A recent study of cardiac mAChR in man using carbon-11- (^{11}C)-labeled methiodide quinuclidinyl benzilate ([^{11}C]MQNB) reported that the physiologically active form of the mAChR could be evaluated by PET (*5*). Although radioiodinated 3-quinuclidinyl benzilate (4-[^{123}I]IQNB) (*6*) has been used to visualize mAChR in the central nervous system (CNS) (*7*), it is not known whether this agent binds to cardiac mAChR. SPECT imaging of mAChR in the human heart has not been demonstrated.

Tritium-labeled dexetimide has been shown to bind to mAChR in rat brain in vitro (*8*) and in vivo (*9*) with high affinity, low nonspecific binding, and slow dissociation rate. The accumulation of dexetimide in the CNS was reported to be stereospecific, saturable, and displaceable. In contrast, the stereoisomer levetimide did not show preferential uptake in regions known to have high concentrations of mAChR, and its affinity was more than a thousand times lower (*4,8,10*). This suggested that levetimide could be used for assessing nonspecific binding. Dexetimide was labeled with ^{11}C (*11*) and an analog has been radiolabeled with iodine-125 (^{125}I) and iodine-123 (^{123}I) (*12*). The in vivo binding of 4-[^{125}I]iododexetimide in the mouse brain was evaluated and shown to be highly specific, saturable, and stereoselective. It was concluded that this ligand may be useful for imaging mAChR in the living human brain with SPECT (*12*).

In the present study, 4-[^{125}I]iododexetimide (Fig. 1A) was investigated as a ligand for cardiac mAChR in the rat to assess the feasibility of using this ligand for the delineation of myocardial mAChR in vivo. The in vitro and the in vivo binding of 4-[^{125}I]iododexetimide to cardiac mAChR was evaluated. In this study, 4-[^{125}I] iodolevetimide (Fig. 1B) was used to determine nonspecific binding.

MATERIALS AND METHODS

Iodine-125-labeled 4-iododexetimide (4-[^{125}I]iododexetimide) and 4-iodolevetimide (4-[^{125}I]iodolevetimide) were syn-

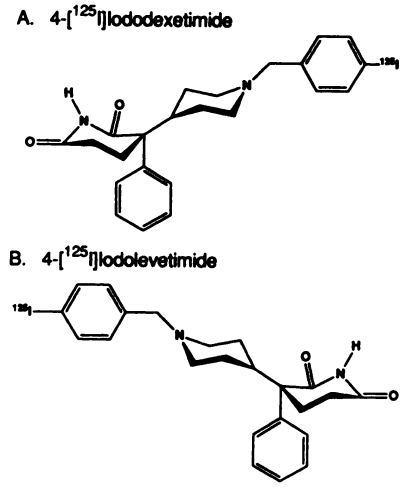

FIGURE 1
Chemical structures of (A) 4-[^{125}I]iododexetimide and (B) 4-[^{125}I]iodolevetimide.

thesized as previously described (*12*). The specific activity of these ligands varied from 300 to 600 Ci/mmol. Atropine sulfate and imipramine hydrochloride were obtained from Sigma Chemical Co., St. Louis, MO.

In Vitro Experiments

Homogenate of ventricle was prepared by excising the rat heart immediately after killing the animal, mincing it with scissors, and rapidly homogenizing the tissue using a Brinkmann Polytron (setting 6, 30 s) in 50 m*M* phosphate-buffered saline (PBS) (pH = 7.4, 37°C). After filtration through four layers of cheese cloth, the homogenate was centrifuged for 15 min at 50,000 g, and the pellet was washed with PBS, recentrifuged, and stored at −80°C. Homogenate of rat atrium was also prepared in similar fashion. Ten minutes before the assay, the tissue homogenates were diluted to 8 mg of tissue/ml with phosphate buffer. Triplicate samples of 0.05 ml of 4-[^{125}I] iododexetimide (final concentration of 0.01 n*M*), and 0.05 ml of increasing concentrations of unlabeled iododexetimide (0.5 n*M*–32 n*M*) were incubated in microfuge tubes with 0.5 ml of the diluted tissue homogenate for 30 min at 37°C in a shaking water bath. The final volume was 1.0 ml. Samples prepared in the presence of 1.0 m*M* atropine defined nonspecific binding.

Following the incubation period, the samples were centrifuged at 10,000 g for 10 min and the pellets were washed with ice cold PBS. The radioactivity concentration in each sample was determined using standard techniques. Net specific binding was determined by subtracting nonspecific binding (samples with atropine) from total binding. Scatchard analysis (*13*) was performed and the dissociation constant (K_d) and the number of binding sites (B_{max}) were calculated using EBDA (*14*) and LIGAND (*15*) programs. Each of the tissues was analyzed in triplicate on four separate days. The protein concentration was measured using the method by Lowry (*16*).

In Vivo Experiments

Male Sprague-Dawley rats (n = 4 per time point; 180–250 g) were injected intravenously via tail vein with 10 μCi of 4-[^{125}I]iododexetimide. The animals were killed by guillotine at various times (15, 30, 60, and 120 min) after i.v. administration of the tracer. Hearts were rapidly removed and dissected into the atrium and ventricle. The lungs and livers were also excised. The radioactivity concentration in tissue samples and an aliquot of the injectate was measured in an automated gamma counter. A second series of rats (n = 4) received 10 μCi of 4-[^{125}I]iodolevetimide and was killed in similar fashion. Blocking experiments (n = 4) were carried out by i.v. injection of atropine sulfate (20 mg/kg) at 5 min before injection of 4-[^{125}I]iododexetimide. The influence of imipramine on 4-[^{125}I] iododexetimide binding was determined by injection of 10 mg/kg imipramine at 30 min before tracer administration (n = 4).

Statistics

Data are presented as means ± s.d. Differences between groups were analyzed using an unpaired t-test. Probability levels of 0.05 or smaller were considered significant.

RESULTS

In Vitro Binding of 4-[^{125}I]iododexetimide to Cardiac mAChR

Figure 2A shows the total and specific binding of 4-[^{125}I]iododexetimide to homogenates of rat ventricle as a function of increasing concentrations of unlabeled iododexetimide. Figure 2B shows an example of a Scatchard plot of 4-[^{125}I]iododexetimide binding to ventricle. Nonspecific binding in the assay, estimated at a concentration of 20 n*M* unlabeled iododexetimide, was high (76% of total binding). A summary of the in vitro binding data is given in Table 1. The dissociation constants (K_ds) for 4-[^{125}I]iododexetimide binding to rat atrium versus that to the ventricle were not significantly different from each other ($p = 0.3$). On the other hand, the B_{max} of the atrium was significantly higher than that of the ventricle ($p < 0.005$).

Biodistribution in Rats

Time-activity curves for 4-[^{125}I]iododexetimide and 4-[^{125}I]iodolevetimide in rat atrium and ventricle at various times after injection are shown in Figures 3A-

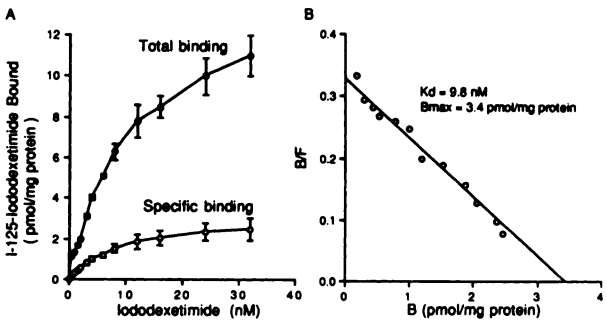

FIGURE 2
(A) In vitro total (—●—) and specific (—○—) 4-[^{125}I]iododexetimide binding as a function of increasing amounts of iododexetimide in the homogenate of rat ventricle. Data shown are the mean of four separate experiments performed in triplicate. Errors shown are standard deviations. (B) Representative Scatchard plot of 4-[^{125}I]iododexetimide binding to the homogenate of rat ventricle.

TABLE 1
Dissociation Constant (K_d) and Maximum Concentration of Binding Sites (B_{max}) of 4-[^{125}I]Iododexetimide Binding to Rat Atrium and Ventricle*

	K_d (n*M*)	B_{max} (pmol/mg protein)
Atrium	11.3 ± 1.7	7.1 ± 1.0
(cv)†	(9.2 – 27.2)	(6.2 – 21.2)
Ventricle	14.0 ± 4.4	3.7 ± 1.2‡
(cv)	(15.4 – 23.8)	(11.1 – 19.5)

* All values represent means ± s.d. for four separate experiments performed in triplicate. Data were calculated using EBDA and LIGAND programs.
† cv = ranges of coefficients of variation (%) calculated for each K_d and B_{max}.
‡ $p < 0.005$.

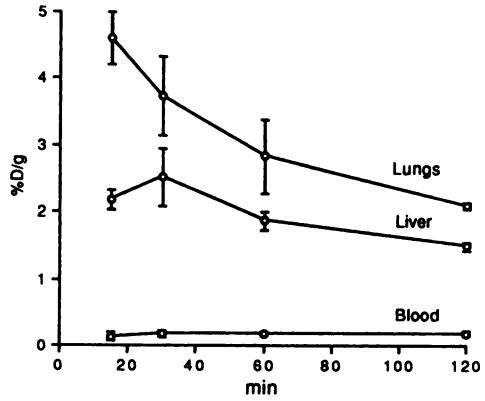

FIGURE 4
Average time-activity curves of 4-[^{125}I]iododexetimide in rat lungs, liver, and blood.

B. Between 15 and 120 min after injection, 4-[^{125}I] iododexetimide concentrations decreased from 2.7% to 1.9 % of the injected dose per gram of tissue (%ID/g) in the atrium and from 1.9 to 1.0 % ID/g in the ventricle. In the blood, the 4-[^{125}I]iododexetimide concentration was relatively low at all time points (0.13–0.18% ID/g) (Fig. 4). At 30 min, the atrium-to-blood ratio was 14.2:1, the ratio for ventricle-to-blood was 10.4:1. In contrast to 4-[^{125}I]iododexetimide, the 4-[^{125}I] iodolevetimide concentration in both the atrium and ventricle was significantly lower and decreased only slightly during the time of observation (15 to 120 min).

For comparison, 4-[^{125}I]iododexetimide concentrations were also followed in the lungs and liver. In both organs (Fig. 4), 4-[^{125}I]iododexetimide concentrations were comparatively high (4.6% ID/g and 2.2% ID/g, respectively, at 15 min and 2.1% ID/g and 1.5% ID/g, respectively, at 120 min after injection). Because of the high lung uptake, heart-to-lung ratios for 4-[^{125}I]iododexetimide were relatively low (0.4–0.9:1). Heart-to-liver ratios were also low (0.7–1.3:1).

To evaluate whether 4-[^{125}I]iododexetimide accumulation in lungs and liver was mAChR-specific, time-activity curves of 4-[^{125}I]iodolevetimide, the inactive enantiomer, were also determined. At all times after injection, the distribution of 4-[^{125}I]iodolevetimide in rat lungs and liver was similar to that of 4-[^{125}I]iododexetimide (data not shown). These results indicate that, although accumulation of 4-[^{125}I]iododexetimide in lungs and liver is high, the binding does not appear to be specific.

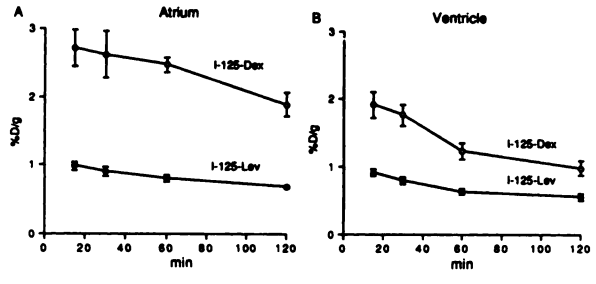

FIGURE 3
Average time-activity curves of 4-[^{125}I]iododexetimide and 4-[^{125}I]iodolevetimide in rat atrium (A), ventricle (B), and blood (A). Values are expressed as means ± S.D. of %ID/g. —○— = 4-[^{125}I]iododexetimide, —●— 4-[^{125}I]iodolevetimide.

In Vivo Blocking Study

To verify the specificity of 4-[^{125}I]iododexetimide binding to cardiac mAChR, inhibition by atropine was studied. As shown in Table 2, the accumulation of 4-[^{125}I]iododexetimide in the rat atrium and ventricle was blocked by prior i.v. administration of a high dose (20 mg/kg) of atropine. The highest inhibition (%ID/g of 4-[^{125}I]iododexetimide minus %ID/g of 4-[^{125}I]iododexetimide with preadministration of atropine) occurred in the atrium (65.6% at 30 min after injection of tracer). In the ventricle, inhibition by atropine averaged 55%.

Previous in vitro studies have shown that cardiac mAChR are blocked by the antidepressant imipramine (*17*). In this investigation, the effect of preadministration of imipramine on the in vivo 4-[^{125}I]iododexetimide binding was determined. Imipramine (10 mg/kg) was injected intravenously 30 min before administration of the ligand. The dose of 10 mg/kg imipramine constitutes approximately twice the maximum dose administered to patients (*18*). Imipramine reduced 4-[^{125}I]iododexetimide accumulation in the rat atrium by 59.5%, in the ventricle by 45.8% (Table 2).

DISCUSSION

In this investigation, the in vitro and in vivo binding of 4-[^{125}I]iododexetimide to rat heart mAChR was studied. In vitro, the 4-[^{125}I]iododexetimide binding was found to be potent (K_d = 11.3 and 14 n*M* in atrium and ventricle, respectively). In vivo distribution studies with 4-[^{125}I]iododexetimide in rats demonstrated that this muscarinic antagonist localizes in the heart and

TABLE 2
4-[^{125}I]Iododexetimide and 4-[^{125}I]Iodolevetimide Accumulation in the Rat Heart

	4-[^{125}I]Iododexetimide (mean %ID/g tissue*)			4-[^{125}I]Iodolevetimide (mean %ID/g tissue*)	
	Controls	Atropine	Imipramine	Controls	Atropine
Atrium	2.62 ± 0.34	0.90 ± 0.13†	1.06 ± 0.04†	0.91 ± 0.13	0.91 ± 0.20
Ventricle	1.79 ± 0.16	0.81 ± 0.08†	0.94 ± 0.02†	0.80 ± 0.07	0.80 ± 0.09

* Results are expressed as mean %ID/g tissue ± s.d. at 30 min after tracer injection; n = 4 rats. Atropine (20 mg/kg) was injected intravenously 5 min before administration of the radiolabeled ligand. Imipramine (10 mg/kg) was injected intravenously 30 min before administration of 4-[^{125}I]iododexetimide.

† Significantly different from controls, $p < 0.001$.

that large target-to-blood ratios are achieved. Kinetic studies with 4-[^{125}I]iododexetimide in the rat heart showed that peak tracer concentrations were reached early (within the first 15 min) after i.v. injection. Thereafter, the radioactivity decreased slowly with an approximate half-time of 150 min. Evidence for the specificity of the interaction of 4-[^{125}I]iododexetimide with mAChR in vivo comes from the results of two experiments: (a) the accmumulation of 4-[^{125}I]iododexetimide in the heart was significantly higher than that of the inactive enantiomer (4-[^{125}I]iodolevetimide, and (b) 4-[^{125}I]iododexetimide accumulation in the heart was inhibited by the muscarinic antagonist atropine. In contrast, 4-[^{125}I]iodolevetimide was not affected by atropine.

In in vivo distribution studies, "specific" binding of 4-[^{125}I]iododexetimide was defined as the difference between total 4-[^{125}I]iododexetimide accumulation and the radioactivity remaining after blockade with a high dose of atropine. "Specific" binding of 4-[^{125}I]iododexetimide was significantly higher in the atrium than in the ventricle. This finding correlated with the in vitro results (B_{max} data shown in Table 1), as well as with previous results in the mouse heart (data not shown). The regional distribution of mAChR seems to vary with different species. In the rabbit heart, as in the rat, highest concentrations of mAChR are found in the atrium (*4*, *19*), the distribution in the guinea pig and dog heart is diffuse (*20*). The organization of mAChR in the human heart is not known for certain. PET studies with [^{11}C] MQNB showed higher tracer concentrations in the ventricular septum and left ventricular wall whereas the atria was not visualized (5). Whether the distribution seen on the PET images is an accurate reflection of receptor densities is not known. Human autopsy data on mAChR distribution in the heart are still outstanding.

Tricyclic antidepressants such as imipramine have been reported to block cardiac mAChR (*17*) and play a role in clinical cardiotoxicity (*21*). In in vivo experiments reported here, the accumulation of 4-[^{125}I]iododexetimide in the heart was significantly blocked by preadministration of imipramine at a dose (10 mg/kg) approximately twice the maximum dose administered to patients. A number of neuroleptics such as chlorpromazine also interact with cardiac mAChR (*4*). Therefore, monitoring of cardiac mAChR during drug intervention could provide a valuable tool in the management and treatment of patients with not only cardiac disease but also with psychiatric disease.

4-[^{125}I]iododexetimide has a high lipophilicity which contributes to the high accumulation in the lungs. In the present investigation, the clearance of 4-[^{125}I]iododexetimide was found to be slow, and the lung activity was more than twice that of the ventricle. High lung uptake was also reported for other iodinated ligands such as 4-[^{125}I]IQNB (*22*) and [^{131}I]iodopindolol for beta-adrenoreceptor (*23*), and may become a major obstacle to the use of these ligands for cardiac receptor imaging. In addition, some pharmaceuticals, such as imipramine, are known to affect uptake of ligands in the lungs (*24*). This fact may have to be taken into consideration in quantitative in vivo receptor studies.

For obtaining a more favorable heart-to-lung ratio, hydrophilic compounds like MQNB may be more useful (*19*). Indeed, [^{11}C]MQNB has been used in in vivo PET scanning of the human heart, in which heart images could be obtained without the need for subtracting lung activity (*5*). Although 4-[^{125}I]iododexetimide exhibits high lung uptake, quantitative SPECT imaging of cardiac mAChR with 4-[^{123}I]iododexetimide should be possible as long as overlying lung activity can clearly be distinguished and separated from that in the atria and ventricles.

Similar considerations have to be made for the possible interference in cardiac imaging by radioactivity accumulated in the liver. 4-[^{125}I]iododexetimide accumulation in the rat liver was about unity with that in the heart. Whether uptake of the ^{123}I-labeled ligand in the human liver would be of equal proportion has to be explored.

The results presented in this study show a high affinity of 4-[^{125}I]iododexetimide for cardiac mAChR. In vivo inhibition studies with atropine, as well as com-

parative studies with the inactive enantiomer, 4-[^{125}I] iodolevetimide, had indicated that ~40% of 4-[^{125}I] iododexetimide binding in the rat heart was nonspecific. In in vitro assays, nonspecific binding in the heart (in the presence of 20 n*M* cold iododexetimide) was even more prominent, amounting to as much as 76% of total binding. A high nonspecific binding (50% of total binding) in the myocardium has also been reported for 4-[^{125}I]IQNB (*22*), which has been attributed to (a) the high lipophilicity of the ligand (b) interactions with contractile cardiac proteins. Whether the same factors or others are responsible for the relatively high nonspecific portion of 4-[^{125}I]iododexetimide binding in the myocardium is not known at present.

CONCLUSIONS

4-[^{123}I]iododexetimide may not necessarily be an ideal ligand for imaging of mAChR of heart because of its high nonspecific binding and low heart-to-lung and heart-to-liver ratios. Nonetheless, an advantage of 4-[^{125}I]iododexetimide is the availability of its labeled inactive enantiomer. Blocking studies for an estimation of nonspecific binding of mAChR in imaging studies of human heart are supposed to be not totally viable, since mAChR antagonists have pronounced pharmacological action which affects cardiac function and may preclude blocking studies. The use of 4-[^{123}I]iodolevetimide should allow an estimation of nonspecific binding in cardiac mAChR imaging studies and measurement of its binding should closely approximate the nonspecific binding component of its enantiomer, 4-[^{123}I]iododexetimide. Since SPECT should allow an in vivo analysis of mAChR in the heart under sympathetic and parasympathetic physiologic control, it is worth considering that this method might represent the more ideal approach to research of the physiologially active form of mAChR. Whether or not successful imaging of mAChR with 4-[^{123}I]iododexetimide will be possible, will depend, in large part, on the kinetics and binding of the tracer in the human heart.

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REFERENCES

1. Narayanan N, Derby JA. Effects of age on muscarinic cholinergic receptors in rat myocardium. *Can J Physiol Pharmacol* 1983;61:822–829.
2. Carrier GO, Aronstam RS. Altered muscarinic receptor properties and function in the heart in diabetes. *J Pharmacol Exp Ther* 1987;242:531–535.
3. Vanter DE, Lee DL, Schwarz KR, et al. Impaired cardiac muscarinic receptor function in dogs with heart failure. *J Clin Invest* 1988;81:1836–1842.
4. Fields JZ, Roeske WR, Morkin E, et al. Cardiac muscarinic cholinergic receptors: biochemical identification and characterization. *J Biol Chem* 1978;253:3251–3258.
5. Syrota A, Comar D, Paillotin G, et al. Muscarinic cholinergic receptor in the human heart evidenced under physiological conditions by positron emission tomography. *Proc Natl Acad Sci USA* 1985;82:584–588.
6. Rzeszotarski WJ, Eckelman WC, Francis BE, et al. Synthesis and evaluation of radioiodinated derivatives of 1-Azabicyclo(2.2.2)oct-3-yl alpha-hydroxy-alpha-(4-iodophenyl)phenylacetate as potential radiopharmaceuticals. *J Med Chem* 1984;27:156–160.
7. Eckelman WC, Reba RC, Gibson RE, et al. External imaging of cerebral muscarinic acetylcholine receptors. *Science* 1984;223:291–293.
8. Laduron PM, Verwimp M, Leysen JE. Stereospecific in vitro binding of [^{3}H]dexetimide to brain muscarinic receptors. *J Neurochem* 1979;32:421–427.
9. Laduron PM, Janssen PFM. Characterization and subcellular localization of brain muscarinic receptors labelled in vivo by [^{3}H]dexetimide. *J Neurochem* 1979;33:1223–1231.
10. Soudijn W., Van Wijngaarden I, Ariëns EJ. Dexetimide, a useful tool in acetylcholine-receptor localization. *Eur J Pharmacol* 1973;24:43–48.
11. Dannals RF, Långström B, Ravert HT, et al. Synthesis of radiotracers for studying muscarinic cholinergic receptors in the living human brain using positron emission tomography: [^{11}C]dexetimide and [^{11}C]levetimide. *Int J Appl Radiat Isot* 1988;39:291–295.
12. Wilson AA, Dannals RF, Ravert HT, et al. Synthesis and biological evaluation of [^{125}I] and [^{123}I]4-iododexetimide, a potent muscarinic cholinergic receptor antagonist. *J Med Chem* 1989;32:1057–1062.
13. Scatchard G. The attractions of proteins for small molecules and ions. *Ann NY Acad Sci* 1949;51:660–672.
14. McPherson GD. A practical computer-based approach to the analysis of radioligand binding experiments. *Computer Prog In Biomed* 1983;17:107–114.
15. Munson PJ, Rodbard D. LIGAND: a versatile computerized approach for the characterization of ligand binding systems. *Anal Biochem* 1980;107:220–239.
16. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–275.
17. Snyder SH, Yamamura HI. Antidepressants and the muscarinic acetylcholine receptor. *Arch Gen psychiatry* 1977;34: 236–239.
18. Davis JM, Glassman AH. Antidepressant drugs. In: Kaplan HI, Sadock BJ, eds. *Comprehensive textbook of psychiatry*, 5th edition. Baltimore: Wiliams & Wilkins; 1989:1627–1655.
19. Gibson RE, Eckelman WC, Vieras F, et al. The distribution of the muscarinic acetylcholine receptor antagonists, quinuclidinyl benzilate and quinuclidinyl benzilate methiodide (both tritiated), in rat, guinea pig, and rabbit. *J Nucl Med* 1979;20:865–870.
20. Wei JW, Sulakhe PV. Regional and subcellular distribution of myocardial muscarinic cholinergic receptors. *Eur J Pharmacol* 1978;52:235–238.
21. Dumovic P, Burrows GD, Vohra J, et al. The effect of tricyclic antidepressant drugs on the heart. *Arch Toxicol* 1976;35:255–262.
22. Gibson RE, Weckstein DJ, Jagoda EM, et al. The characteristics of I-125-4-IQNB and H-3-QNB in vivo and in vitro. *J Nucl Med* 1984;25:214–222.
23. Hughes B, Marshall DR, Sobel BE, et al. Characterization of beta-adrenoreceptors in vivo with iodine-131-pindolol and gamma scintigraphy. *J Nucl Med* 1986;27:660–667.
24. Moretti JL, Holman BL, Delmon L, et al. Effect of antidepressant and narcoleptic drugs on n-isopropyl-p-iodoamphetamine biodistribution in animals. *J Nucl Med* 1987;28:354–359.