Comparison of Technetium-99m Aminoalkyl Diaminodithiol (DADT) Analogs as Potential Brain Blood Flow Imaging Agents

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N-ethyl piperidinyl diaminodithiol (NEP-DADT), complexed with ^{99m}Tc has been developed as an agent for the measurement of brain blood flow using SPECT. Studies in patients have shown that ^{99m}Tc NEP-DADT enters rapidly into the brain, but also clears rapidly ($t_{1/2}$ = 17 min). In this study nine new aminoalkyl DADT derivatives were synthesized, labeled with ^{99m}Tc and tested in mice with the aim of developing an agent with increased retention in the brain. In addition, relationships between chemical properties of the derivatives and their in vivo localization were investigated. The results were as follows: (a) the R-group and its isomeric configuration has a profound influence on the biodistribution; (b) ^{99m}Tc aminoalkyl DADT derivatives with apparent pK_a values of >6.9 show poor brain uptake (<0.40 % dose at 5 min); (c) lengthening of the chain between the DADT moiety and the amino-R group from ethyl to hexyl generally increases the apparent pK_a and consequently lowers brain uptake; (d) a correlation (r = 0.71) exists between initial brain uptake and the octanol-buffer partition coefficient; (e) ^{99m}Tc-4′-methyl NEP-DADT has the highest partition coefficient, relatively high uptake, and longest retention in the mouse brain. This complex has characteristics suited for brain blood flow measurements.

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The technetium-99m (^{99m}Tc) complex of N-ethyl piperidinyl diaminodithiol ([^{99m}Tc]NEP-DADT) has recently been developed in our laboratory (*1*). This agent has good qualities for single photon emission computed tomography (SPECT) imaging of brain blood flow in experimental animals (*1,2*) and normal volunteers (*3*). However, because of its relatively fast clearance from the brain, it cannot be considered optimal. Present day SPECT imaging technology with rotating cameras calls for prolonged brain retention, preferably up to 1 hr, as well as a fixed distribution during the time of the study (*4*). In order to evaluate the possibility of increasing uptake and retention of the ^{99m}Tc-labeled complex in the brain, several derivatives of [^{99m}Tc]DADT were prepared and tested in mice.

Five new analogs of the N-aminoethyl and four analogs of the N-aminohexyl DADT complex were synthesized, labeled with ^{99m}Tc and separated by high performance liquid chromatography (HPLC) into two respective components (A and B). Their biodistribution in mice was compared with that of [^{99m}Tc]NEP-DADT (A and B). An attempt was made at correlating chemical structure against in vivo localization in order to aid in the custom design of future brain blood flow imaging agents of similar structure.

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MATERIALS AND METHODS

Preparation of the DADT Complex and its Analogs

The following ligands as the tri-HCl salts were prepared in an analogous fashion to the procedure for NEP-DADT (*1*): N-ethylpiperidinyl DADT (NEP-DADT, I, see Fig. 1); N-ethyl N,N′-dimethylamino DADT (II); N-ethyl 4′-methylpiperidinyl DADT (III); N-ethyl 4′-propylpiperidinyl DADT (IV); N-ethylmorpholinyl DADT (V); N-ethylisopropylamino DADT (VI); N-hexylpiperidinyl DADT (VII); N-hexyl N,N′-dimethylamino DADT (VIII); N-hexyl morpholinyl DADT (IX) and N-hexyl isopropylamino DADT (X). Each ligand was characterized by IR, NMR, and elemental analysis. The analytic data was consistent with the assigned structures.

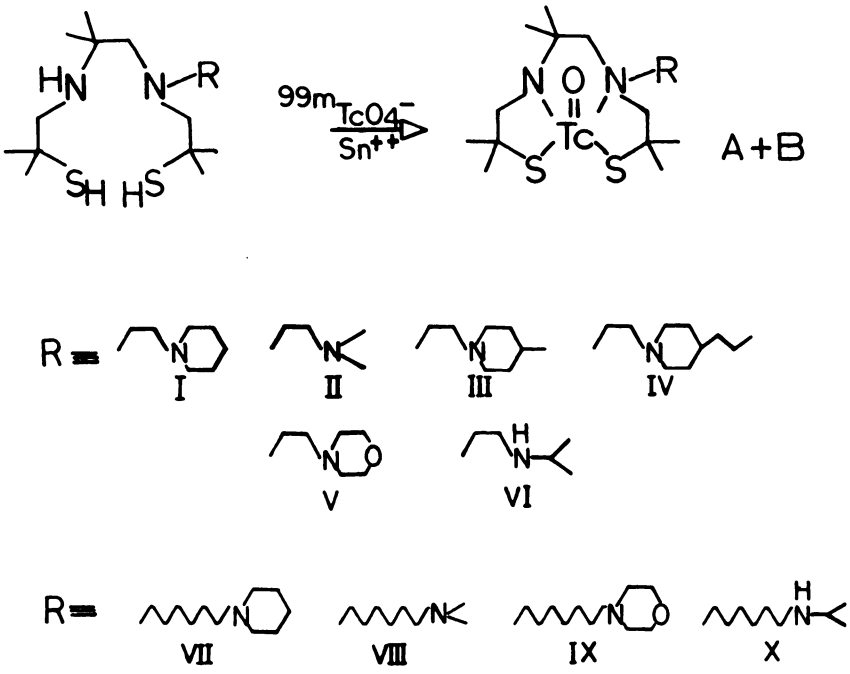


FIGURE 1
Labeling of aminoethyl (I-VI) and aminohexyl (VII–X) DADT ligands with [^{99m}Tc]pertechnetate in the presence of $SnCl_2$. In each case two major complexes (A and B) were formed.

Tc-99m Labeling and Isolation of Complexes

Labeling of the different ligands with ^{99m}Tc was performed as previously described in detail (*1*). In brief, to a solution of 1 mg of ligand in 0.4 ml of pH 7.1 phosphate buffer was added a solution of stannous chloride dihydrate in ethanol (0.1 ml, 1.33×10^{-4} *M*), followed by 5–10 mCi sodium [^{99m}Tc]pertechnetate in 0.1–0.3 ml isotonic saline. All reactions were carried out at room temperature for 15 min, except for ligands III and IV that were heated at 50°C for 30 min.

Separation and purification procedures depended on the type of ligand. The ^{99m}Tc aminoethyl analogs (Complexes I–VI) were first extracted into hexane then separated by normal phase HPLC using a silica gel column* and methylene chloride:ethanol (98:2) as solvent system. The two main peaks (A and B, where B eluted first) were collected in separate flasks and five milliliters of isotonic saline were added to each fraction. Subsequently, the organic phase was removed by evaporation under vacuum without heat. The ^{99m}Tc aminohexyl derivatives (Complexes VII–X), on the other hand, were separated on a reverse phase HPLC column† with acetonitrile:0.01 N aqueous ammonium acetate (45:55) as the solvent mixture. In this system peak A eluted before peak B. After isolation, the fractions were subjected to rotoevaporation under vacuum for removal of acetonitrile. The purity of each complex was determined in the following manner. For complexes isolated by normal phase HPLC, the collected fractions were re-analyzed by normal phase HPLC and silica gel thin layer chromatography‡ (95:5 CH_2Cl_2:EtOH). Complexes IV-A and IV-B were analyzed by HPLC only. For complexes isolated by reverse phase HPLC, the collected fractions were re-analyzed by reverse phase HPLC. The purity was found to be >95% for all complexes except the following: I-B (90%), IX-A (70%), and IX-B (86%). The contaminant in these cases eluted near the void volume on reverse phase HPLC and probably consists of salts of [^{99m}Tc] pertechnetate generated by partial decomposition of the complexes during the isolation procedure. For use in biodistribution studies all HPLC separated ^{99m}Tc complexes were diluted to a concentration of 10 μCi/ml with isotonic saline.

Determination of Apparent Ionization Constant

The apparent pK_a of each of the ^{99m}Tc complexes was estimated by measuring their octanol-buffer distribution coefficient (O-B DC) at various pH values. For this purpose, 10–100 μl (~5 μCi) of the ^{99m}Tc complex were introduced into a 100 mm × 10 mm screw capped tube containing 3 ml of either HCl/KCl (0.05–0.2*M*) at pH 1.0–2.2, 0.1*M* citric acid/citrate at pH 3.0–6.0, 0.2*M* phosphate buffer at pH 6.0–7.5, or 0.1*M* TRIS at pH 7.0–9.9. For each of the complexes O-B D.C. determinations were done in triplicate at 12 to 14 different pH values. Three milliliters octanol were added and each tube was gently shaken on an automatic mixing apparatus for 5 min. After a 5-min centrifugation at 1,500 *g*, 1 ml aliquots of the octanol and the buffer phase were transferred into a plastic tube and counted in an automatic gamma scintillation counter.§ The counting error was kept below 3%. O-B DCs were plotted against pH. The apparent pK_a values were estimated from the curves by two methods: derivative analysis by locating the pH of maximum difference in O-B DCs and the Henderson-Hasselbach equation using the O-B DC for protonated and unprotonated species.

Determination of Octanol-Buffer Partition Coefficient

Octanol-buffer partition coefficients were measured by introducing 0.2–0.5 ml of each of the ^{99m}Tc complexes into isotonic phosphate buffer (*5*) at pH 7.4 (total volume: 3.0 ml), and then adding 3 ml of 1-octanol. After shaking the mixture for 5 min and centrifuging for 3 min, the supernatant octanol phase was transferred into another tube and reequilibrated with 3 ml of fresh buffer four more times. After the fifth partitioning, two milliliter samples of each phase were removed and the ^{99m}Tc activity in buffer and octanol deter-

mined. The partition coefficient was obtained by calculating the ratio of net CPM/ml of octanol to that of buffer.

Determination of Protein Binding

Protein binding was evaluated by ultrafiltration. Thirty to two hundred microliter aliquots of solutions of the ^{99m}Tc complexes (10–15 μCi) were added to 3 ml of pooled human serum and incubated for 15 min at room temperature. One milliliter serum samples were transferred to Centricon-30 microconcentrators[¶] with >95% membrane retention for molecules above 30,000 D. Centrifugation was performed at 4,000 *g* for 20 min at 4°C using a fixed 34° angle rotor. Adsorption to the membrane was measured by counting the filter after removal of filtrate and serum concentrate. Less than 5% of the ^{99m}Tc activity was found with the filter membrane. Technetium-99m diethylenetriaminepentaacetic acid (DTPA), known not to bind to serum proteins (*6*), was recovered to 100% in the filtrate under similar incubation and filtering conditions.

Biodistribution Studies

Male CD-1 mice[**] weighing 25–35 g were injected intravenously into the tail vein with 0.2 ml (~2 μCi) of each of the ^{99m}Tc-labeled DADT analogs. At various times after injection, the animals were killed. Immediately before death, a blood sample (0.1 ml) was collected from the jugular vein into a glass capillary which was then placed into a counting tube filled with 0.9 ml water. Organs of interest (brain, heart, lungs, liver, spleen, kidneys, intestines) were dissected, weighed, and prepared for counting. The ^{99m}Tc radioactivity was measured in an autogamma scintillation counter. The percent of radioactivity in the whole blood was estimated using a blood volume of 7% of the body weight. The average recovery of ^{99m}Tc radioactivity at 5 min after injection, i.e., the sum of the percentages of the dose found in all organs including the carcass was 99.5 ± 3.1% (mean of 15 determinations ± 1 s.d.).

RESULTS

Figure 1 shows six different aminoethyl and four aminohexyl DADT ligands which were prepared in our laboratory and labeled with Sn^{2+} reduced [^{99m}Tc]pertechnetate. Each of the formed ^{99m}Tc complexes was separated on HPLC. The two main constituents of the HPLC separation designated A and B, represent, most likely, geometric isomers of the complexes as shown for [^{99m}Tc]NEP-DADT (*7,8*). Confirmation for the complexes in this series is still in progress.

The organ distribution in mice for the different ^{99m}Tc aminoethyl- and aminohexyl DADT analogs is presented in Tables 1A and 1B. The analogs are ordered according to their brain uptake at 5 min after i.v. injection. Technetium-99m NEP-DADT (Complex I, peak A) and ^{99m}Tc N-ethyl-N,N′-dimethylamino DADT (Complex II, peak A) had the highest brain uptake (1.95 and 1.94% of the injected dose, respectively), followed by ^{99m}Tc 4′-methyl NEP-DADT (Complex III, peak A) (1.48% dose). Brain/blood ratios for these three compounds were also appreciably higher (4.28, 3.97 and 3.18, respectively) than all others.Lengthening the chain length from ethyl to hexyl between the DADT moiety and the amino R-group significantly lowered brain uptake as demonstrated by three out of the four hexyl analogs tested. The exception was the ^{99m}Tc aminohexyl morpholino derivative that, in the A form, showed similar uptake in the brain as the aminoethyl morpholino derivative. In the B-form, it demonstrated increased uptake (1.14% vs. 0.61%, $p < 0.005$).

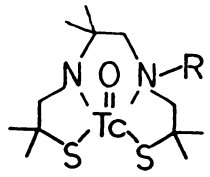
All [^{99m}Tc]DADT analogs showed considerable accumulation in liver and gastrointestinal tract. Lung uptake was variable, ranging from 0.88% to 11.21% of the injected dose. However, no relationship between in vitro lipophilicity, as determined by the octanol-buffer partition coefficient at pH 7.4, and lung uptake was observed.

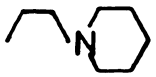
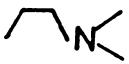
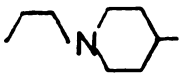
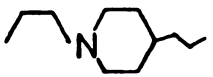
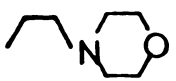
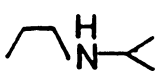
Technetium-99m activity in brain and other major organs was followed for a 2-hr period for complexes which had demonstrated high initial brain uptake, i.e., for [^{99m}Tc]NEP-DADT (Complex I, peaks A and B), ^{99m}Tc-N-ethyl N,N′-dimethylamino DADT (Complex II, peaks A and B) and ^{99m}Tc 4′-methyl NEP-DADT (Complex III, peaks A and B). Brain and blood curves for four of these complexes were selected to be presented in Figure 2. Technetium-99m NEP-DADT (I A) and ^{99m}Tc N-ethyl N,N′-dimethylamino DADT (II, A and B) disappeared rather rapidly from the brain. In contrast, ^{99m}Tc 4′-methyl NEP-DADT (III A) brain activity remained relatively high over the entire observation period, falling from 3.1 ± 0.17% dose/g (mean of 12 determinations ± 1 s.e.m.) at 5 min after injection to 1.74 ± 0.08% dose/g at 60 min and to 1.19 ± 0.11% dose/g at 120 min. Blood clearance of ^{99m}Tc 4′-methyl NEP-DADT (III A) was rapid, resulting in high brain/blood ratios (3.2 at 5 min to 4.6 at 2 hr after injection).

The biodistribution data for Complexes I, II, and III (peaks A and B) at 30 and 60 min after injection are listed in Table 2. Most striking was the rapid accumulation of ^{99m}Tc activity in liver and GI tract. The combined uptake in these two organs ranged from 60–70% of the injected dose at 30 min to 70–79% at 60 min. The majority of the remaining dose was found in the carcass (data not shown). The results indicate that these lipophilic ^{99m}Tc complexes are excreted primarily via the biliary system and the gut.

Several physicochemical properties of the different ^{99m}Tc aminoethyl and aminohexyl DADT complexes were examined in order to investigate their relationship with biological localization. Drug entry into the brain is governed, according to Rapoport et al. (*9*), by three main factors: (a) its lipophilicity, (b) its degree of ionization at the pH of blood, and (c) its binding to serum proteins. Accordingly, the octanol-water partition coefficient, apparent dissociation constant and protein binding of each of the ^{99m}Tc complexes was determined.

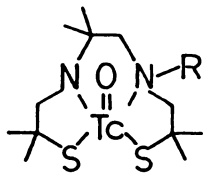
TABLE 1A
Biodistribution of [^{99m}Tc]Aminoethyl-Diaminodithiol Complexes



R	Complex	(n)	Concentration of Complexes in Tissues % D/organ mean ± 1 s.d. at 5 min after injection							
			Brain	Blood	Heart	Lungs	Liver	Kidneys	GI	Brain/blood*
	I A	(6)	1.95 ±0.19	1.89 ±0.18	0.77 ±0.21	6.16 ±0.89	13.35 ±2.34	6.12 ±0.81	14.54 ±0.87	4.28 ±0.29
	I B	(6)	1.11 ±0.15	3.69 ±0.61	0.42 ±0.05	2.09 ±0.83	22.15 ±2.95	4.13 ±0.76	18.12 ±2.41	1.41 ±0.22
	II A	(9)	1.94 ±0.32	2.08 ±0.14	0.45 ±0.08	8.10 ±1.95	15.67 ±2.15	7.51 ±1.00	13.59 ±2.24	3.97 ±0.34
	II B	(9)	1.43 ±0.19	2.94 ±0.37	0.41 ±0.05	3.57 ±0.69	22.24 ±2.29	4.29 ±1.12	16.62 ±2.17	1.86 ±0.27
	III A	(9)	1.48 ±0.20	1.90 ±0.49	1.02 ±0.17	3.66 ±0.71	15.11 ±3.05	5.51 ±0.59	14.28 ±2.62	3.18 ±0.35
	III B	(6)	1.15 ±0.20	2.65 ±0.52	0.47 ±0.09	1.73 ±0.35	19.32 ±2.31	4.97 ±0.91	10.20 ±1.43	2.12 ±0.49
	IV A	(6)	0.74 ±0.14	3.07 ±0.38	0.57 ±0.05	2.92 ±0.66	26.83 ±2.31	3.93 ±0.78	15.32 ±2.91	1.02 ±0.21
	IV B	(6)	1.00 ±0.20	3.68 ±0.59	0.92 ±0.25	2.48 ±0.74	26.16 ±3.26	4.53 ±0.49	13.69 ±2.39	1.24 ±0.30
	V A	(6)	0.69 ±0.03	3.30 ±0.05	0.36 ±0.02	0.89 ±0.20	24.03 ±4.53	2.37 ±0.27	24.47 ±2.30	0.88 ±0.07
	V B	(5)	0.61 ±0.10	3.90 ±0.64	0.36 ±0.05	0.88 ±0.13	23.14 ±4.69	2.21 ±0.21	22.73 ±3.73	0.67 ±0.05
	VI A	(6)	0.47 ±0.12	1.72 ±0.17	0.56 ±0.06	11.21 ±1.52	14.64 ±1.93	8.49 ±1.22	16.22 ±1.53	1.15 ±0.15
	VI B	(6)	0.88 ±0.11	2.74 ±0.98	0.45 ±0.05	8.06 ±1.30	17.45 ±0.93	7.42 ±1.59	15.84 ±1.99	1.60 ±0.68

*$\frac{\%\ D/g\ brain}{\%\ D/ml\ blood}$

TABLE 1B
Biodistribution of [^{99m}Tc]Aminohexyl-Diaminodithiol Complexes



R	Complex	(n)	Concentration of Complexes in Tissues % D/organ mean ± 1 s.d. at 5 min. after injection							
			Brain	Blood	Heart	Lungs	Liver	Kidneys	GI	Brain/blood*
	VII A	(6)	0.10 ±0.02	2.93 ±0.57	0.42 ±0.05	5.38 ±1.64	31.10 ±4.54	7.17 ±0.64	23.69 ±5.67	0.18 ±0.05
	VII B	(6)	0.18 ±0.06	2.88 ±0.46	0.53 ±0.11	7.21 ±2.22	27.63 ±5.42	6.91 ±0.68	22.05 ±4.09	0.35 ±0.10
	VIII A	(6)	0.12 ±0.01	2.13 ±0.32	0.60 ±0.04	7.39 ±1.48	15.22 ±2.29	8.71 ±0.81	19.14 ±1.59	0.11 ±0.02
	VIII B	(6)	0.18 ±0.03	1.94 ±0.38	0.68 ±0.09	7.02 ±1.15	16.29 ±1.15	7.66 ±1.15	19.02 ±0.84	0.10 ±0.01
	IX A	(9)	0.68 ±0.17	4.02 ±1.86	0.47 ±0.11	3.56 ±1.07	14.83 ±4.75	3.82 ±1.59	20.58 ±8.40	0.79 ±0.30
	IX B	(9)	1.14 ±0.32	3.40 ±0.52	0.63 ±0.08	5.65 ±1.32	14.51 ±2.30	5.25 ±0.74	22.39 ±2.06	1.34 ±0.25
	X A	(6)	0.05 ±0.01	1.92 ±0.30	0.68 ±0.13	3.18 ±0.53	23.64 ±4.34	9.14 ±2.80	22.35 ±3.34	0.11 ±0.02
	X B	(6)	0.06 ±0.01	2.43 ±0.30	0.75 ±0.15	3.98 ±0.89	22.15 ±2.23	9.84 ±1.12	22.35 ±3.04	0.10 ±0.01

* $\frac{\%\ D/g\ brain}{\%\ D/ml\ blood}$

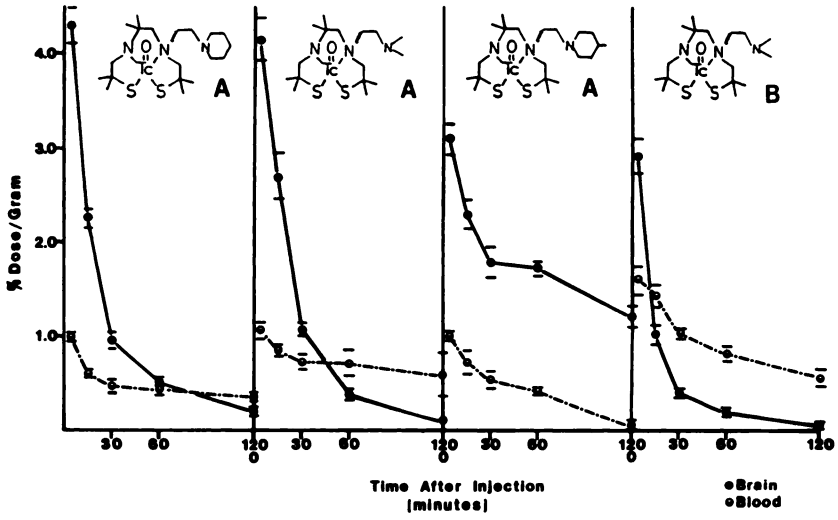


FIGURE 2
Brain and blood clearance curves of [^{99m}Tc]NEP-DADT (I A), ^{99m}Tc N-ethyl N,N′ dimethylamino DADT (II A and B), and ^{99m}Tc 4′-methyl NEP-DADT (III A). Technetium-99m complex III A showed longest activity retention in the brain and highest brain to blood ratios.

Octanol partition coefficients for the different DADT derivatives at pH 7.4 ranged between 11 and 1,671 (log P = 1.04 − 3.22) (Table 3). When the log P data were plotted against percent brain uptake at 5 min after i.v. injection (Fig. 3), a correlation of r = 0.71 was observed, indicating at least to a certain extent, a linear relationship between entry of the ^{99m}Tc complex into the brain and its distribution between octanol and buffer at the pH of blood. Furthermore, it was interesting to note that the complex with the highest lipophilicity (log P = 3.22) was the 4′-methyl-NEP-DADT (III) complex which had been identified as having the longest retention in the brain (Fig. 2). The apparent ionization coefficients were determined by measuring the octanol-buffer distribution of the ^{99m}Tc complexes various pH-values and constructing a curve, similar to a titration curve. The apparent pK_a's ranged between 4.1 and 7.5 (Table 3). Earlier experiments with the parent complexes had indicated that the dissociation curves did not represent equilibria of the coordinated amines. Technetium-99m complexes with apparent pK_a of >6.9 showed poor brain uptake (<0.40% dose at 5 min after injection). This latter finding agreed with the postulate that ionized species pass the blood–brain barrier either poorly or not at all (*10*). In addition, it was observed that peak A of the aminoethyl complexes (that always predominates in our labeling procedure) exhibited a higher apparent pK_a than peak B. In the aminohexyl complexes, on the other hand, where the electron density of the amine is insulated from the complex core, no difference was seen.

Protein binding, as determined by ultrafiltration ranged between 86% and 99% for all [^{99m}Tc]DADT complexes (Table 3). In spite of this high degree of binding, some of the complexes exhibited relatively high initial brain uptake after intravenous injection (see Table 1). This leads to the conclusion that the on and off kinetics of binding must occur relatively fast; in particular, that these complexes are readily dissociated from serum proteins in order to be able to penetrate the blood–brain barrier immediately after intravenous injection.

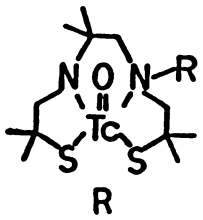
DISCUSSION

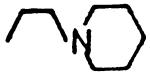
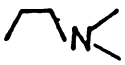
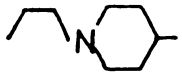
Several laboratories have directed research efforts towards the development of ^{99m}Tc-labeled agents suitable for evaluation of cerebral blood flow by SPECT imaging, a goal first stated by Oldendorf (*11,12*).

One ligand system which forms neutral, lipid soluble complexes with ^{99m}Tc is propylene-amine-oxime (PAO). It was first described by Troutner et al. (*13*) and Volkert et al. (*14*) in 1983. Studies with the original complex in human beings showed relatively low first-pass extraction and lack of retention in the brain (*15*) and, therefore, led to the search for a better agent. Amersham International synthesized and tested over 100 analogs of the PAO ligand (*16*), of these d-1 HM-PAO was selected. Several groups of investigators have reported encouraging results using [^{99m}Tc]HM-PAO in patients with various neurologic disorders (*17–19*). However, the [^{99m}Tc]HM-PAO complex is not without problems: its in vitro stability is rather limited (see supplier's instruction sheet) and, in vivo, it decomposes extremely fast after intravenous injection, leading to relatively high levels of ^{99m}Tc activity remaining in the blood stream (*2,20*).

Another of the ligands developed was the diaminodithiol (DADT) ligand that forms neutral, lipid soluble ^{99m}Tc (V) complexes (*21,22*) and has been shown to cross the blood–brain barrier (*23*). Kinetic studies of several unsubstituted [^{99m}Tc]DADT complexes have shown rapid transport into the brain, but clearance from the brain occurred within a few minutes. Increased brain uptake as well as longer retention has been demonstrated when a piperidinylethyl side chain was at-

TABLE 2
Biodistribution of [^{99m}Tc]Aminoethyl-Diaminodithiol Complexes



Complex	Concentration of Complexes in Tissues % D/organ mean ± 1 s.d. at 30 and 60 min after injection													
	Brain		Blood		Heart		Lungs		Liver		Kidneys		GI	
	30′	60′	30′	60′	30′	60′	30′	60′	30′	60′	30′	60′	30′	60′
 I A	0.48 ±0.11	0.24 ±0.03	0.93 ±0.22	0.78 ±0.26	0.31 ±0.04	0.19 ±0.04	0.92 ±0.27	0.54 ±0.09	26.32 ±3.02	22.06 ±3.34	1.41 ±0.31	1.03 ±0.21	40.02 ±3.93	53.58 ±2.67
I B	0.17 ±0.03	0.08 ±0.02	1.70 ±0.28	1.29 ±0.26	0.12 ±0.01	0.08 ±0.01	0.55 ±0.04	0.33 ±0.08	26.61 ±2.74	26.49 ±3.06	1.94 ±0.41	1.37 ±0.13	43.19 ±4.99	52.49 ±7.06
 II A	0.50 ±0.08	0.17 ±0.04	1.41 ±0.24	1.28 ±0.30	0.14 ±0.02	0.08 ±0.02	2.14 ±0.61	1.02 ±0.29	25.36 ±0.82	19.88 ±1.01	2.68 ±0.33	1.57 ±0.24	36.35 ±3.06	51.94 ±5.22
II B	0.19 ±0.02	0.09 ±0.02	1.66 ±0.15	1.36 ±0.30	0.10 ±0.02	0.07 ±0.01	1.07 ±0.10	0.61 ±0.19	23.45 ±2.27	22.26 ±3.72	1.54 ±0.26	1.29 ±0.53	41.93 ±3.95	53.90 ±3.51
 III A	0.86 ±0.14	0.82 ±0.08	1.09 ±0.30	0.81 ±0.06	1.12 ±0.29	1.05 ±0.32	1.19 ±0.37	0.74 ±0.14	23.60 ±6.98	28.33 ±2.04	1.71 ±0.54	1.12 ±0.14	33.35 ±5.44	41.17 ±4.70
III B	0.69 ±0.08	0.49 ±0.05	1.81 ±0.17	1.06 ±0.20	0.94 ±0.09	0.79 ±0.14	0.95 ±0.13	0.58 ±0.08	23.16 ±0.59	21.11 ±2.19	1.55 ±0.24	1.15 ±0.20	45.17 ±1.18	53.54 ±3.18

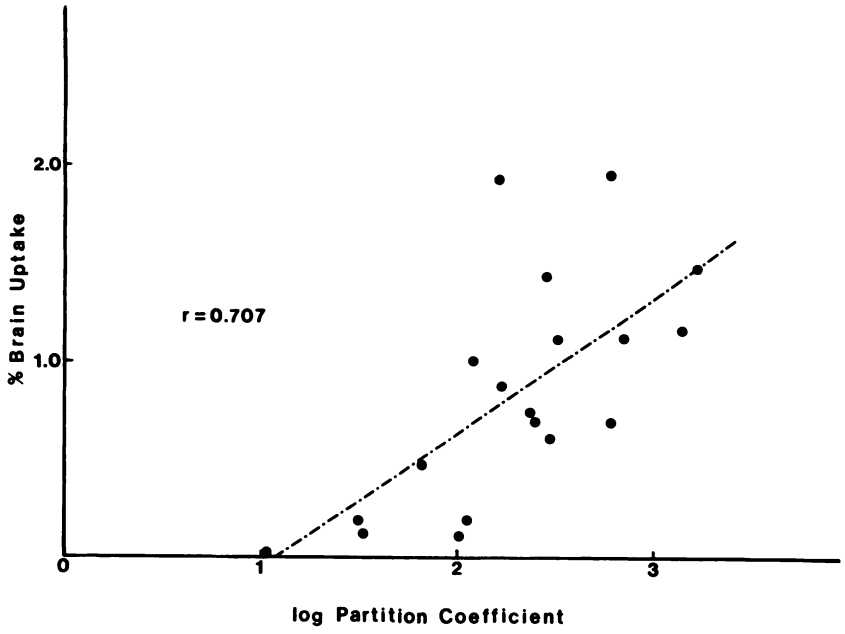
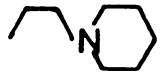
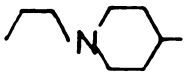
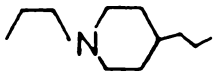
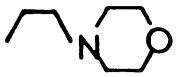
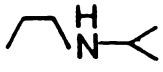


FIGURE 3
Relationship between in vitro lipophilicity of the [^{99m}Tc]DADT complexes and their initial uptake in the mouse brain. The lipophilicity was determined by measuring the octanol-buffer coefficients at pH 7.4. Brain uptake was determined as percent of the dose at 5 min after injection.

tached to the DADT ligand (N-ethyl piperidinyl-DADT, (NEP-DADT)) (*1*).

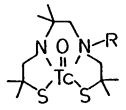
Preliminary studies with [^{99m}Tc]NEP-DADT in human volunteers have shown that washout of the complex from the brain occurs with a half-time of 17 min (*3*). Since comparatively long (5–10 min per rotation) imaging times with present-day SPECT cameras are required to accumulate adequate counts for measure-

TABLE 3A
Physicochemical Properties of [^{99m}Tc]Aminoethyl-Diaminodithiol Complexes

R	Complex	Partition coefficient (log P)*	Apparent pK_a†	Percent serum binding‡
	I A	2.78	5.3	96.8
	I B	2.52	4.4	97.4
	II A	2.21	6.1	93.8
	II B	2.41	5.4	92.8
	III A	3.22	5.5	97.7
	III B	3.14	4.7	98.3
	IV A	2.38	4.3	99.5
	IV B	2.08	4.0	99.0
	V A	2.40	5.1	92.2
	V B	2.47	4.1	89.2
	VI A	1.82	7.1§	91.9
	VI B	2.23	6.7	96.8

* Octanol-buffer partition coefficient at pH 7.4.
† Measured by determination of octanol-buffer distribution coefficient at various pH values.
‡ Determined by ultrafiltration.
§ Partially ionized at pH 7.4.

TABLE 3B
Physicochemical Properties of [^{99m}Tc]Aminohexyl-Diaminodithiol Complexes



R	Complex	Partition coefficient (log P)*	Apparent pK_a†	Percent serum binding‡
	VII A	2.04	7.0§	97.9
	VII B	2.03	7.5§	96.8
	VIII A	1.53	6.9§	94.7
	VIII B	1.50	6.9§	93.4
	IX A	2.79	5.2	92.2
	IX B	2.84	5.3	89.2
	X A	1.04	6.9§	93.0
	X B	1.04	6.9§	96.0

* Octanol-buffer partition coefficient at pH 7.4.
† Measured by determination of octanol-buffer distribution coefficient at various pH values.
‡ Determined by ultrafiltration.
§ Partially ionized at pH 7.4.

ments of brain blood flow, a ^{99m}Tc agent with longer brain residence time and little or no redistribution could be highly desirable. As advances in SPECT imaging technology continue, this requirement will become less stringent. In fact, the use of agents with a short residence time could be utilized for repetitive or perturbation studies on the same day.

The purpose of our present investigation was (a) to study different [^{99m}Tc]DADT derivatives as to their distribution and retention in the brain and (b) to gain insight into the relationship between structure of the complex and its biological function with the goal to develop a better agent for the measurement of brain blood flow by SPECT imaging.

All complexes studied were lipophilic with octanol-buffer (pH 7.4) coefficients log $p > 1.0$, thus, according to Oldendorf (*24*), freely diffusible through the blood–brain barrier. However, another criterion has to be met for a lipophilic agent to diffuse through the blood–brain barrier: it has to remain unionized at physiological pH (*25*). Some of the [^{99m}Tc]DADT complexes in our series were found to be partially ionized and hence, showed poor brain uptake (<0.4% dose in mouse brain at 5 min after injection). The [^{99m}Tc]DADT complexes that demonstrated the lowest brain uptake (<0.2% dose at 5 min postinjection), were the aminohexyl DADT derivatives; all had apparent pK_a values of 6.9 or greater. Serum protein binding was essentially the same for all [^{99m}Tc]DADT complexes tested. Whether the relatively high binding determined by ultrafiltration was due to specific protein binding or to adsorption of the lipophilic agents to serum components was not evaluated. A direct relationship between the amount of ^{99m}Tc complex taken up in the brain and serum protein binding could not be demonstrated.

Of the complexes tested in mice ^{99m}Tc 4′-methyl NEP-DADT showed the slowest clearance from the brain. SPECT studies in dogs, comparing ^{99m}Tc 4′-methyl NEP-DADT with [^{99m}Tc]NEP-DADT demonstrated no significant difference between the two ^{99m}Tc complexes (*2*). In contrast, recent SPECT studies in the same baboon showed a clear advantage of the methylated complex: brain clearance was prolonged, on an average, about two times. Reasons for the interspecies differences are presently under investigation.

NOTES

*(Micro-Porasil, 10 μ; 8 mm I.D., Waters Z module cartridge) Waters Co., Milford, MA.

†(4 μ Nova Pak, 8 mm I.D. Waters Z module) Waters Co., Milford, MA.

‡(Machery-Nagel) Brinkmann Instruments, Westbury, NY.

§Packard Instrument Co., Inc., Downers Grove, IL.

¶Amicon, Danvers, MA.

**Charles River Laboratories, Wilmington, MA.

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