PREPARATION AND BIOLOGICAL EVALUATION OF TECHNETIUM-99m-PHENYLETHYLAMINE COMPLEXES

Arturo A. Vitale[a*#], María Ana Calviño[ab], Carina C. Ferrari[ab], Adriana E. Stahl[a] and Alicia B. Pomilio[a*]

[a]PROPLAME-CONICET, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina, [b]INEUCI-CONICET, Departamento de Ciencias Biológicas, FCEN, UBA, Argentina.

SUMMARY.- Biological and chemical characteristics of ^{99m}Tc-phenethylamines complexes are presented. 2-(4,5-Dimethoxy-2-nitrophenyl)ethylamine, 2-(3,4-dimethoxyphenyl)ethylamine and 2-(2-amino-4,5-dimethoxyphenyl)-ethylamine were used as ligands. A preliminary evaluation of these ^{99m}Tc-complexes as dopamine receptor radioligands was also performed. Net charges at each atom were also calculated by a semiempirical ZINDO/1 method for comparison of free ligands' parameters with those of the respective technetium-complexes.

Keywords: 2-(4,5-Dimethoxy-2-nitrophenyl)ethylamine; 2-(3,4-Dimethoxyphenyl)-ethylamine; 2-(2-Amino-4,5-dimethoxyphenyl)ethylamine; 99m-Technetium complexes; Preparation; Biodistribution; semiempirical ZINDO/1 calculations.

INTRODUCTION

The sympathetic nervous system is vitally involved in the homeostatic regulation of a wide variety of functions. Agents that mimic or alter its activity are useful in the treatment of several clinical disorders, including hypertension, shock, cardiac failure and arrhythmias, asthma, allergy and anaphylaxis (1). As might be expected, sympathomimetic amines, which are structurally substituted phenethylamines, such as naturally occurring

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* Research Members of the National Research Council of Argentina (CONICET).

\# To whom any inquiries should be addressed.

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catecholamines, *e.g.* noradrenaline, adrenaline, dopamine, and drugs that mimic these actions, such as amphetamines, comprise one of the more extensively studied groups of pharmacological agents. The role of the catecholamines in the central nervous system (CNS) is also relevant, as it appears likely that most of the pathological states of the brain (including functional disorders, such as schizophrenia and maniac-depressive psychoses) manifest themselves as abnormalities in amine metabolism or altered receptor density.

In the radiopharmaceutical chemistry attempts are continuously being made to replace the ^{123}I, ^{131}I, ^{67}Ga, and ^{111}In-labelled compounds with the corresponding ^{99m}Tc-compounds because of the poor physical characteristics, high production costs and/or limited availability of the former (2). As ^{99m}Tc has none of these disadvantages, several investigators have sought to develop new tracers based upon this radionuclide.

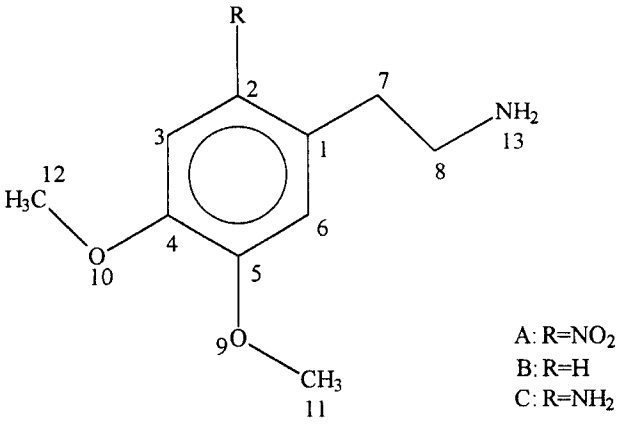
^{123}I-Labelled phenethylamines were prepared for brain studies in rats (3), and derivatives of phenethylamines possessing antiadrenaline activity were synthesized replacing C, N and/or I with radionuclides (4). As far as we know there are no reports on ^{99m}Tc-phenethylamines complexes. Thus, this paper describes the biological and chemical characteristics of three substituted phenylethylamines complexes with ^{99m}Tc. A preliminary evaluation of these ^{99m}Tc-complexes as dopamine receptor radioligands was also performed.

MATERIALS AND METHODS

Preparation and identification of Ligands. Compounds **A** [2-(4,5-Dimethoxy-2-nitrophenyl)ethylamine] and **C** [2-(2-amino-4,5-dimethoxyphenyl)ethylamine] were synthesized according to literature methods. (5). Compound **B** [2-(3,4-dimethoxyphenyl)ethylamine] is commercially available (Fluka). The purity of compounds **A**, **B** and **C** was assessed by HPLC (LKB chromatograph equipped with a LKB Bromma 2249 solvent-delivery pump, a Rheodyne injector and a variable-wavelength Pharmacia LKB-VWM 2141 UV detector coupled to a LKB Bromma 2221 integrator) using a RP-C18 column (μBondapak C18, 10 μm, 250 mm length x 4.6 mm i.d. column) (methanol-water, 80:20; 1 ml/min) and UV-detection at 254 nm. Structures were determined by ^{1}H- and ^{13}C-NMR spectroscopy using a Bruker AC-200 spectrometer operating at 200 and 50.2 MHz, respectively, and the molecular weights by high-resolution mass spectrometry (HRMS) using a ZAB-SEQ (U.K.) instrument. The FT-IR (Mattson 3000 FT-IR spectrometer Unicam) spectra were also recorded. Chemical structures of the ligands are shown in Fig. 1.

Computational methods. All computational studies were performed using the Hyperchem package (Autodesk, version 3, hardware lock Nr. N-102802, Prof. A. B. Pomilio).

Figure 1 : Structures of ligands



Molecular geometries of the ^{99m}Tc-**A**, -**B**, and -**C** complexes and of the ligands **A**, **B** and **C** were optimized by the semiempirical ZINDO/1 method. The minimization was carried out until the rms of gradients was less than 0.01 kcal/mol Å. Net charges were obtained using the Hyperchem ZINDO/1 method with the Polak-Ribiere optimization algorithm. The semiempirical ZINDO/1 method is the modified version of INDO/1 including parameters for the transition metals, *e.g.* technetium (6, 7).

Technetium complexation. Sodium pertechnetate-^{99m}Tc was obtained from a commercial ^{99}Mo/^{99m}Tc generator (Medgenix Diagnostics). The ^{99m}Tc-complex of each phenylethylamine was prepared as follows:

Solution A: The corresponding phenylethylamine (Comp. **A** = 1 mg; Comp. **B** = 40 mg; Comp. **C** = 6-10 mg) was added to a physiological saline solution (1 ml) and heated if necessary until complete dissolution.

Solution B: Sodium tartrate (80 mg) and stannous chloride (0.2 mg) were added to a physiological saline solution (2 ml) and heated until complete dissolution.

Solution C: Three drops of solution B were added to solution A and the pH was adjusted to 10. Then 185 MBq (5 mCi) of $^{99m}TcO_4^-$ were added to solution C, and the final volume was adjusted to 2 ml with physiological saline solution. The reaction mixture was stirred and sonicated for 10 min at room temperature.

Analysis of ^{99m}Tc-complexes. The distribution of radioactivity on chromatograms was quantitatively analysed using an ionization chamber detector. Complex radiochemical purity (CRP) was assayed by thin layer chromatography. Test samples were applied on neutral alumina plates (1 x 10 cm aluminium backed strips) and immediately developed by ascending chromatography using ethanol (96%) as mobile phase. After development, the strips were dried and the radioactivity distribution was determined.

Electrophoresis. The charge of the complexes was determined by electrophoresis using a Bio Rad power supply, 50 mM phosphate buffer adjusting to different pH's (7.0, 7.4, 8.0 and 11) and at 300 V for 30 min and 1 h, and 450 V for 30 min under nitrogen atmosphere and control of temperature. Whatman N° 1 electrophoresis strips (1 cm x 30 cm) were soaked in the respective phosphate buffers for at least 30 min in an electrophoresis bath before spotting the sample. Each ^{99m}Tc-complex was applied to the strips, which were placed in an electrophoresis chamber containing the buffer (200 ml) and run under the conditions mentioned above. After drying, the strips were cut into 2 cm sections and counted in a Clinigamma counter.

Biodistribution of ^{99m}Tc-complexes. Biodistribution studies were performed in female Sprague-Dawley rats (200-300 g) in groups of three. Each animal was anaesthetized by i.p. injection of sodium thiopental (60 mg/kg). Then it was placed in a supine position and the radiopharmaceutical (0.2 ml) was i.c. bolus injected. At different times post-administration (5, 15, 30 and 90 min) animals were sacrificed by cervical disarticulation. At killing, samples of blood were collected in preweighed containers. Liver, stomach, small intestines, large intestines, heart, lungs, spleen, brain, adrenals, kidney and bladder with urine were counted with an automatic gamma detector (Clinigamma Pharmacia). The accumulated activity in each organ or tissue was calculated as a percentage of the total dose, determined by a dose calibrator (Vexal). For blood, the calculation was based upon the measured activity and weight of the sample, and body composition data (6%).

Regional brain uptake. The brains were removed, placed on ice, and dissected into the cerebellum and striatum. These regions were weighed and counted using a gamma detector. The uptake ratio was obtained by dividing percent dose/g of the striatum with that of the cerebellum.

RESULTS AND DISCUSSION

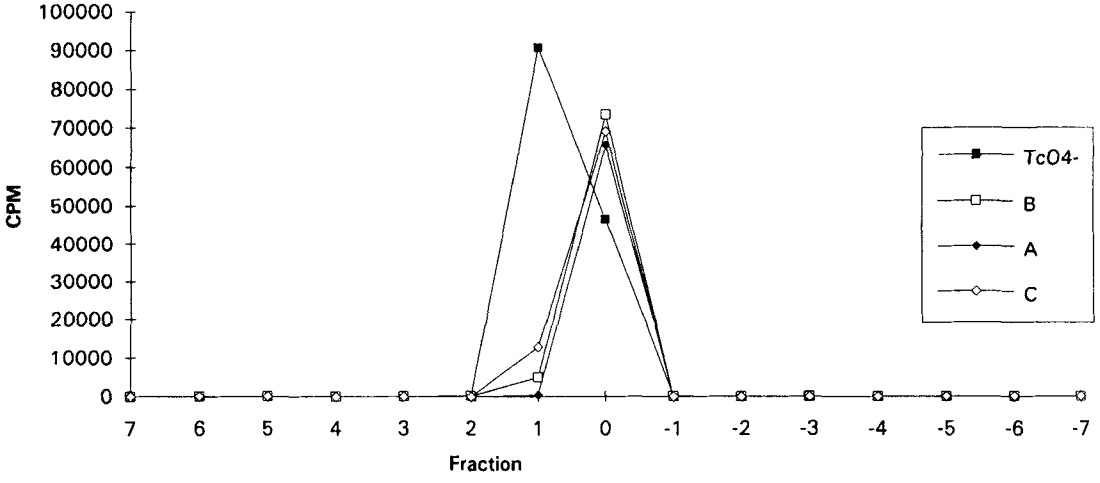
Radiochemical purity (RCP). All preparations gave more than 90% radiochemical yield.

Chromatography of the ^{99m}Tc-phenethylamine complexes of **A, B** *and* **C**. A good separation of the ^{99m}Tc-**A**, -**B**, and -**C** complexes was achieved by thin layer chromatography (Tc^{2+}: R_f= 0; TcO_4^-: R_f = 0.7; complexes: R_f = 1.0).

Characterization of the complexes. Structural characterization of technetium compounds by classical means requires the use of long-lived ^{99}Tc ($t_{1/2}$ = 2.10^5 years). The ^{99}Tc complexes were prepared and identified by FAB-MS and FT-IR. Additionally, crystals of these 99-Tc complexes suitable for X-ray analysis are being prepared to solve the respective structures. HPLC analysis has shown the correspondence between ^{99}Tc and ^{99m}Tc complexes. Details of the spectral analysis and characterization of these complexes will be the subject of a separate paper.

Electrophoresis. The electrophoresis of the ^{99m}Tc-**A**, -**B**, and -**C** complexes and pertechnetate showed that the complexes stayed at the origin as evidence that they are neutral while the charged pertechnetate (as -1 species at pH 7.0 and 7.4) moved towards the anode, as expected. Fig. 2 shows the total activity (cpm) versus the fraction, confirming that pertechnetate is not present in the complexes.

Figure 2 : Electrophoresis at 450v , 30 m.

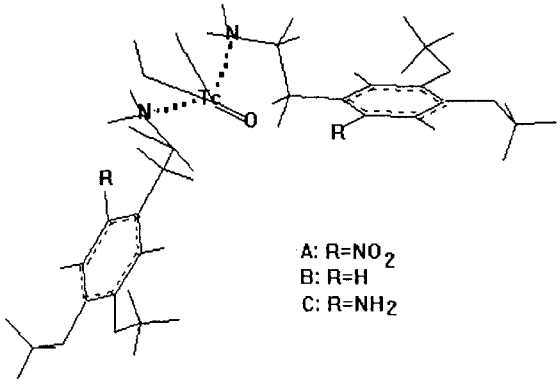


The stability of the complexes was studied at different pH (7.0 to 11.0), and different times (*e.g.* 30 min vs 1 h), comparing results under nitrogen vs. air exposure. According to electrophoresis results, the relative stability of ^{99m}Tc complexes is: ^{99m}Tc-**B** > ^{99m}Tc-**C** > ^{99m}Tc-**A**. *Oxidation to pertechnetate* was the only decomposition product detected. As expected, the relative stability is directly related to the inductive effect of the substituents at the aromatic ring.

Moreover, the same electrophoresis behaviour was observed for the three complexes at blood pH (7.4) and brain tissue pH (7.0), suggesting the occurrence of the same neutral lipophilic complexes under the *in vivo* conditions. Accordingly, they may diffuse freely across the blood-barrier brain and as complexes cannot be easily retained and diffuse out.

Net charges calculations. Net charges for each atom of the ^{99m}Tc-**A**, -**B**, and -**C** complexes and of the ligands **A**, **B** and **C** were calculated in their probable biologically active conformations to evaluate them as potential dopamine receptor ligands. Values are shown in Table 1, and minimal energy conformations of Tc-**A**, -**B** and -**C** are shown in Fig 3. Calculated data are not significantly altered by the coordination with the Tc atom. As it is

Figure 3: Calculated conformations of technetium compounds



known, the congruent molecular electrostatic potential (MEP) minima represent a part of dopaminergic pharmacophore (8) and are directly related to net charges. For comparative reasons net charges were used in this case because of the Technetium parameters' requirement.

These three ligands have in common the 3,4-dimethoxyphenethyl structure so that the three-dimensional pharmacophore may be similar for the three compounds, except for the effect of the substituents at position 2 of the aryl group. According to previous *ab initio*, ^{13}C- and ^{17}O-NMR studies in hydroxy- and methoxybenzene derivatives that we have performed (9-14), both methoxy groups prefer a conformation in which the methyls are located in the same plane as the benzene ring, and each methoxy group pointing away from each other. Regions of important negative charge were found in the proximity of the lone pairs of the heteroatoms. One region is found in the proximity of the amine nitrogen, while another important region is found in the proximity of both oxygens of the 3,4-dimethoxy groups. These characteristics are in agreement with those common to all dopaminergic ligands. As reported (15, 16) the minima generated by the aromatic ring is less intense and modulated by its substituents.

However, the MEP is unlikely to account, on its own, for the differences in D_1/D_2 selectivity, but it could be a subtle modulator of affinity as recently (15) reported. Controversy has arisen (16) concerning this viewpoint.

Furthermore, these electrostatic interactions differ depending on the direction of the interaction between a phenyl group and another polar group, as has been previously calculated (17). The interaction between a benzene ring and a positively charged species is most favourable if the positive species interacts with the benzene ring above or below the ring plane. On the other hand, the interaction between a benzene ring and a negatively charged species

is most favourable if the negative species is located in the plane of the benzene ring. The polar groups interacting with the aryl ring can be possible receptor sites, such as a sp3-hybridized cationic NH_3 group, or an anionic carboxy group. A negative charge density implies an energetically favourable interaction between the molecule and the charged receptor site.

The aryl group could interact with the D_2 receptor requiring electron-rich π rings, such as charge transfer or dispersion interactions. Such interaction appears not to be so important for the D_1 ligands. Substituents (OCH_3, NH_2, NO_2) modulate the MEP values in the aryl region, thus modifying its electron density.

To this point, the discussion has been based upon the compounds' relative affinity to the dopamine (D_1 and/or D_2) receptors. However, dopaminergic compounds have been known to bind to other biogenic amine receptors (α_1, α_2, β, $5HT_{1a}$, $5HT_{1c}$ and $5HT_2$).

Biodistribution. Biodistribution results of ^{99m}Tc-**A**, -**B**, and -**C** complexes are presented in Tables 2 to 4, where values of the percent uptake at 5, 15, 30 and 90 min post-administration are shown.

Comparing the three complexes studied, ^{99m}Tc-**A** showed the lowest brain uptake values. ^{99m}Tc-**B** showed at 5 min an uptake maximum of 0.10% of the dose administered, progressively falling with the time. ^{99m}Tc-**C** presented the greater uptake, approx. 0.10 and 0.12%, which remained constant throughout the study (1.5 hs). This last point is important, since the capacity of a tracer of staying in the brain with a fixed distribution for a minimum time of 20 to 30 min is a requirement for obtaining good image acquisition in a SPECT.

However, the complexes tested were not able to reach moderate brain uptake values, such as a 0.4% of the administered dose (18, 19), and much less a 'good initial uptake' that would imply values near to 1% (19-22).

With respect to the behaviour of the complexes in the liver, the uptake was in the order: ^{99m}Tc-**A**, -**B**, and -**C** (approximately the double of the previous), confirming the major lipophilicity of ^{99m}Tc-**C**, also evidenced in the highest lung uptake values.

Furthermore, in the case of ^{99m}Tc-**B** a possible hepatic clearance was evidenced by the marked decrease in the uptake during 15 and 30 minutes coinciding with the simultaneous rise in the thin intestine.

Concerning the distribution of the three complexes in the blood, it could be correlated with that in the brain, which may be taken as proof of the availability of the complexes for blood-brain exchange. It must be noted that the reversible binding of the pharmaceuticals to plasmatic proteins can act as a pool that slowly releases the active agents (20).

It is evident that the ^{99m}Tc-**C** complex is maintained for longer and in higher proportion in the blood possibly due to a greater affinity for blood cells or plasmatic proteins than the other complexes studied.

Table 1. Net atomic charges of compounds

At.	Z	A-Tc-A	A	B-Tc-B	B	C-Tc-C	C
1	6	0.024527	0.067356	0.064046	0.055015	-0.019894	-0.037668
2	6	0.030173	-0.001040	-0.072577	-0.072116	0.146838	0.172810
3	6	-0.122964	-0.087554	-0.083627	-0.085238	-0.156657	-0.176653
4	6	0.171233	0.160098	0.148262	0.144946	0.174348	0.178919
5	6	0.147316	0.175081	0.170112	0.165112	0.136210	0.118940
6	6	-0.106217	-0.123219	-0.129475	-0.129109	-0.099247	-0.094360
7	6	-0.096940	-0.080415	-0.038584	-0.082796	-0.032048	-0.063464
8	6	0.319430	0.058702	0.202163	0.078972	0.164617	0.075469
9	8	-0.236151	-0.235276	-0.240873	-0.243742	-0.240270	-0.245131
10	8	-0.239372	-0.235259	-0.240109	-0.243530	-0.239869	-0.244959
11	6	0.039150	0.038657	0.040017	0.040912	0.040324	0.042083
12	6	0.039464	0.037216	0.040017	0.040442	0.039865	0.041239
13	7	-0.441962	-0.341093	-0.416968	-0.345097	-0.437474	-0.345823

Table 2. Biodistribution of complex **A**

	% dose/organ[1]			
ORGAN	**5 min**	**15 min**	**30 min**	**90 min**
heart	0.29 ±0.02	0.20 ±0.05	0.17 ±0.09	0.11 ±0.00
lungs	1.18 ±0.38	2.38 ±1.58	1.13 ±0.41	0.72 ±0.15
liver	3.04 ±0.28	2.82 ±0.61	2.95 ±0.35	3.05 ±1.35
stomach	3.43 ±2.10	3.66 ±0.68	5.75 ±1.91	7.29 ±0.48
small int.	2.35 ±0.43	3.16 ±1.00	3.45 ±1.09	2.62 ±0.21
large int.	1.00 ±0.09	1.08 ±0.31	1.65 ±0.32	1.91 ±0.41
spleen	0.43 ±0.11	0.37 ±0.04	0.39 ±0.06	0.20 ±0.02
adrenals	0.03 ±0.01	0.02 ±0.01	0.02 ±0.00	0.01 ±0.00
kidneys	0.86 ±0.13	0.80 ±0.29	0.77 ±0.14	0.61 ±0.05
bladder	0.61 ±0.69	0.45 ±0.32	0.75 ±0.44	3.10 ±0.69
brain	0.05 ±0.01	0.04 ±0.02	0.04 ±0.01	0.03 ±0.00
blood[2]	11.68 ±0.24	9.71 ±2.83	11.22 ±2.87	6.33 ±0.11

Table 3. Biodistribution of complex **B**

	% dose/organ[1]			
ORGAN	**5 min**	**15 min**	**30 min**	**90 min**
heart	0.76 ±0.43	0.44 ±0.12	0.26 ±0.02	0.51 ±0.16
lungs	2.21 ±0.69	1.49 ±0.16	1.42 ±0.60	0.92 ±0.27
liver	4.84 ±0.69	4.74 ±0.12	4.15 ±0.30	3.76 ±1.35
stomach	3.86 ±0.74	5.25 ±1.47	3.81 ±1.43	3.49 ±0.64
small int.	4.01 ±0.69	3.89 ±1.19	4.51 ±0.87	3.34 ±0.15
large int.	2.10 ±0.45	2.15 ±0.47	2.43 ±0.16	2.96 ±0.05
spleen	0.47 ±0.08	0.35 ±0.08	0.54 ±0.20	0.16 ±0.01
adrenals	0.05 ±0.01	0.06 ±0.02	0.03 ±0.00	0.03 ±0.01
kidneys	1.11 ±0.12	1.25 ±0.06	0.93 ±0.04	0.99 ±0.04
bladder	0.17 ±0.01	0.22 ±0.08	1.10 ±0.33	1.80 ±0.03
brain	0.10 ±0.03	0.08 ±0.01	0.05 ±0.00	0.05 ±0.01
blood[2]	19.11 ±3.73	14.35 ±2.94	11.92 ±0.88	9.70 ±1.23

Table 4 Biodistribution of complex **C**

	% dose/organ[1]			
ORGAN	**5 min**	**15 min**	**30 min**	**90 min**
heart	0.76 ±0.05	0.46 ±0.05	0.49 ±0.17	0.32 ±0.08
lungs	2.69 ±1.10	2.77 ±0.08	2.39 ±0.42	1.90 ±0.41
liver	6.35 ±0.76	9.36 ±0.17	9.13 ±2.53	9.26 ±1.14
stomach	2.11 ±0.18	2.40 ±0.30	5.82 ±0.61	5.17 ±1.27
small int.	2.76 ±0.27	3.46 ±0.84	3.70 ±1.13	5.90 ±1.80
large int.	2.09 ±0.53	2.82 ±0.46	2.30 ±0.31	2.85 ±0.68
spleen	0.93 ±0.14	0.91 ±0.09	1.08 ±0.20	0.96 ±0.38
kidney	2.46 ±0.42	2.33 ±0.10	2.32 ±0.73	2.04 ±0.52
bladder	0.40 ±0.09	1.39 ±0.23	1.36 ±0.33	3.58 ±1.13[2]
brain	0.11 ±0.03	0.10 ±0.02	0.12 ±0.01	0.10 ±0.01
blood[2]	16.60 ±1.10	17.27 ±2.00	22.24 ±1.75	19.09 ±1.60

[1] Average of three rats, mean ± SD.
[2] See experimental

In all three cases good renal clearance is observed after 90 min.

Regional brain uptake. One of the ways of determining whether a ligand is *in vivo* linked to receptors is testing the correlation between the regional labelling distribution and the distribution of the receptors. However, it is

important to examine the accumulation and rate of washout of radioactivity from the striatum, a region of rat brain expressing a high density of D2 receptors (23). Therefore, we analysed the striatum:cerebellum ratio, which is a measure of the specific: nonspecific binding of a dopamine-based radioligand *in vivo*. The content of the complex in the cerebellum is a measure of its nonspecific binding in the brain, while the content in the striatum accounts for the total ligand, that is specific and nonspecific bound ligand (24, 25). It is expected furthermore that the ratio increases significantly while it will be greater the greater the elapsed post-injection time, since the ligand only will remain bound where the interaction with the receptor will be specific (19, 24, 26-30).

The striatum /cerebellum ratio of ^{99m}Tc-**A** increased with time: 1.87 and 2.50 at 15 min and 90 min, respectively. However, the complex was washed out only in a moderate way from cerebellum, whereas in striatum it showed major retention. These results suggest a moderate selectivity of these complexes for D_2 dopamine receptor binding, and affinity for other receptors with nonspecific association.

It is worth mentioning that several drugs containing the substructure of 3,4-dimethoxyphenethylamine, such as the verapamil-like drugs, impair the movement of calcium through the voltage-dependent channels and are referred to as organic calcium antagonists. These drugs are used to treat hypertension, angina, cardiac arrhythmias, and migraine headaches. Also cations similar to Tc, such as manganese, mimic the physiological actions of Ca at low concentrations but block Ca at higher concentrations (31). Numerous antischizophrenic neuroleptic drugs act allosterically at the verapamil site. Although these drugs are thought to exert their therapeutic actions by blocking dopamine receptors, at therapeutic doses Ca channels in the brain should be influenced by them. Moreover, centrally active Ca antagonists lacking dopamine blockers might also exert therapeutic behavioural effects (32, 33).

CONCLUSIONS

In the present paper we report on the preparation of some substituted phenylethylamines-^{99m}Tc complexes for the first time. Charge determination and relative stability of the complexes was studied by electrophoresis under different experimental conditions, showing that the three complexes are neutral and their relative stability is: ^{99m}Tc-**B** > ^{99m}Tc-**C** > ^{99m}Tc-**A**.

Biodistribution data and an example of a complementary test commonly used with radiopharmaceuticals at the brain receptors research area but this time using a Tc complex are also presented herein.

Net charges for ^{99m}Tc-**A**, -**B**, and -**C** complexes and for ligands **A**, **B**, and **C** have been calculated using the semiempirical ZINDO/1 method that includes technetium parameters in order to obtain an understanding of the nature of the

interactions between the phenyl ring and the receptor. Based on this study, the conclusion is drawn that an important part of the interaction is due to electrostatic forces (34), where the oxygen atoms of both aromatic methoxyls have a dominant contribution.

Additional structural and biological studies of such complexes are currently in progress.

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