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Technepine: A High-Affinity ^{99m}Technetium Probe to Label the Dopamine Transporter in Brain by SPECT Imaging

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KEY WORDS Dopamine, Cocaine, Parkinson's disease, SPECT imaging, WIN 35,428 or CFT

INTRODUCTION

Increasing evidence suggests that the dopamine transporter, localized on dopamine neurons, is a marker for a number of physiological and pathological states (Kaufman and Madras, 1991, 1993; Madras et al., 1990a, Schoemaker et al., 1985; Singer et al., 1991). With the development of sensitive probes, brain imaging and measurement of the transporter have become feasible in recent years (Brownell et al., in press; Innis et al., 1991; Frost et al., 1993; Madras et al., 1991; Morris et al., submitted; Seibyl et al., 1995; van Dyke et al.,1995; Wong et al., 1993, 1995). Drugs of many chemical classes, including cocaine, bind to the dopamine transporter (Seeman, 1993). Nevertheless, effective imaging agents have been developed almost exclusively from the phenyltropane analogue of cocaine WIN 35,428 or CFT, a potent dopamine transport inhibitor (Clarke et al., 1973; Heikkila et al., 1979). The impetus for developing [^{11}C]WIN 35,428 as a PET ligand (Hantraye et al., 1992; Madras, 1994; Madras et al., 1991, 1994; Wong et al., 1993; Meltzer et al., 1993) and γ-emitting analogues for SPECT imaging (e.g., RTI-55, the 4-iodophenyl analogue of WIN 35,428, Canfield et al., 1990; Boja et al., 1991; Innis et al., 1991) arose directly from our observations of the binding of WIN 35,428 to the dopamine transporter. Unlike previous dopamine transport inhibitors (noncocaine congeners) proposed for brain imaging (Kuhar et al.,1990), the radiolabeled form of WIN 35,428 binds to the dopamine transporter in brain striatum with very low levels of nonspecific binding (Madras et al., 1989a,b) and distributes principally to dopamine-rich regions of brain, as we reported in 1989 (Canfield et al., 1989) and subsequently (Canfield et al., 1990; Kaufman et al., 1991; Kaufman and Madras, 1992).

SPECT imaging techniques are more practical than PET for routine clinical studies because of the lesser technical and logistical burden. Both ^{123}I and ^{99m}Tc are radionuclides suitable for SPECT imaging, but the requirements for attaching these to a target molecule are very different. The relative ease of introducing a radioactive iodine label on the aromatic ring of WIN 35,428 thus resulted in a ^{123}I analogue probe of WIN 35,428 (Boja et al., 1991; Carroll et al., 1992; Innis et al., 1991).

N-Allyl and *N*-propyl analogues of WIN 35,428 (Madras et al., 1990b, 1992) offered the potential for placing γ-emitting isotopes on *N*-substituents. Based on this structure, the agent Altropane (*N*-iodoallyl-2β-carbomethoxy-3β-(4-fluorophenyl)tropane) was recently developed and found to display pharmacokinetic and binding properties highly favorable for SPECT imaging (Fischman et al., submitted; Madras, 1992; Madras et al., 1995).

A systematic study of arylalkyl substituents at the tropane nitrogen showed that bulky groups do not markedly reduce binding affinity for the dopamine transporter (Meltzer et al., in press). This and the effec-

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Abbreviations: ^{99m}Tc, ^{99m}technetium; WIN 35,428 or CFT, 2β-carbomethoxy-3β-(4-fluorophenyl)tropane; WIN 35,140, 2α-carbomethoxy-3β-(phenyl)tropane; dichloropane, 2β-carbomethoxy-3β-(3,4-dichlorophenyl)tropane; difluoropine, (S)-(+)-2β-carbomethoxy-3α-[bis(4-fluorophenyl)methoxy]tropane; O-861R, (RS)-[N-(2-((3′-*N*′-propyl-(1″*R*-3″β-(4-fluorophenyl)tropane-2″β-carbomethoxy))(2-mercaptoethyl)amino)acetyl)-2-aminoethanethiolato]rhenium (V) oxide; Technepine or O-861T, (*RS*)-[*N*-(2-((3′-*N*′-propyl-(1″*R*-3″β-(4-fluorophenyl)tropane-2″β-carbomethoxy))(2-mercaptoethyl)amino)acetyl)-2-aminoethanethiolato]-technetium (V) oxide; O-862R, (*RS*)-[*N*-(2-((3′-*N*′-propyl-(1″*R*-3″β-(4-fluorophenyl)tropane-2″α-carbomethoxy))(2-mercaptoethyl)amino)acetyl)-2-aminoethanethiolato]rhenium (V) oxide; O-863R, (*RS*)-[*N*-(2-((3′-*N*′-propyl-(1″*R*-3″β-(3,4-dichlorophenyl)tropane-2″β-carbomethoxy))(2-mercaptoethyl)amino)acetyl)-2-aminoethanethiolato]rhenium (V) oxide; O-864R, (*RS*)-[*N*-(2-((3′-*N*′-propyl-(1″*S*-3″α-((bis(4-fluorophenyl)methoxytropane-2″β-carbomethoxy))(2-mercaptoethyl)-amino)acetyl)-2-aminoethanethiolato]rhenium (V) oxide; SPECT, single photon emission computed tomography; PET, positron emission tomography.

tiveness of altropane in primate imaging studies led us to explore the feasibility of introducing ^{99m}Tc into phenyltropanes by linking the chelated metal to the nitrogen. Using the nonradioactive congener element rhenium as the metal, a series of analogues of WIN 35,428 were synthesized and their binding to the dopamine transporter investigated (Meltzer et al., in preparation). Rhenium is the congener of technetium in group VIIa of the periodic table and may be used as a model.

We now report the first ^{99m}Tc agent capable of imaging the dopamine transporter. The binding properties of one of the compounds (O-861R) was found to fall well within the range of values observed with other imaging agents for the dopamine transporter. This phenyltropane analog was prepared with ^{99m}Tc as the metal, rather than rhenium. By SPECT imaging in monkey, the compound technepine was found to cross the blood-brain barrier and to localize selectively in the striatum.

MATERIALS AND METHODS

Tissue sources and preparation

Brain tissue from adult male and female cynomolgus monkeys (*Macaca fascicularis*) was stored at −85°C in the primate brain bank at the New England Regional Primate Research Center. The caudate-putamen was dissected from coronal slices and yielded approximately 1.4 g tissue. The thalamus was dissected from coronal slices and tissue from two brains was pooled (1 g). Membranes were prepared as described previously (Madras et al., 1989b).

Dopamine transporter assay

The dopamine transporter was labeled with [^{3}H]WIN 35,428 ([^{3}H]CFT, 2β-carbomethoxy-3β-(4-fluorophenyl)-*N*-[^{3}H]methyltropane, 81.04 Ci/mmol, DuPont-NEN). The affinity of novel compounds for the dopamine transporter was determined in experiments by incubating tissue with a fixed concentration of [^{3}H]WIN 35,428 and a range of concentrations of the compound as described previously (Madras et al., 1989b).

Serotonin transporter assay

The serotonin transporter was assayed in caudate-putamen membranes using conditions similar to those for the dopamine transporter. The affinity of drugs for the serotonin transporter labeled by [^{3}H]citalopram (spec. act.: 81.86 Ci/mmol, DuPont–NEN) was determined in experiments by incubating tissue with a fixed concentration of [^{3}H]citalopram and a range of concentrations of drug. The 2-h incubation (0–4°C) was initiated by addition of membranes and terminated by rapid filtration over Whatman GF/B glass fiber filters presoaked in 0.1% polyethyleneimine. The filters were washed twice with 5 ml Tris–HCl buffer (50 mM), and the remaining steps were carried out as described above. Total binding was defined as [^{3}H]citalopram bound in the presence of ineffective concentrations of unlabeled citalopram (1 pM) or the test compounds. Nonspecific binding was defined as [^{3}H]citalopram bound in the presence of an excess (10 μM) of fluoxetine. Specific binding was the difference between the two values.

Norepinephrine transporter assay

The norepinephrine transporter was assayed in thalamus membranes using conditions similar to those for the serotonin transporter and adapted from the assay for whole rat brain (Gehlert et al., 1995). The affinity of [^{3}H]nisoxetine (spec. act.: 74 Ci/mmol, DuPont–NEN) for the norepinephrine transporter was determined in experiments by incubating tissue with a fixed concentration of [^{3}H]nisoxetine and a range of concentrations of unlabeled nisoxetine. The assay tubes received the following constituents at a final assay concentration: nisoxetine or drug (0.2 ml; 1 pM–300 μM), [^{3}H]nisoxetine (0.2 ml; 0.6 nM); membrane preparation (0.2 ml; 4 mg original wet wt of tissue/ml). The buffer in the assay medium was Tris–HCl: 50 mM, pH 7.4 at 0–4°C; NaCl 300 mM. The 16-h incubation at 0–4°C was initiated by addition of membranes and terminated by rapid filtration over Whatman GF/B glass fiber filters presoaked in 0.3% polyethyleneimine for 1 h. The remaining steps are described above. Total binding was defined as [^{3}H]nisoxetine bound in the presence of ineffective concentrations of drug. Nonspecific binding was defined as [^{3}H]nisoxetine bound in the presence of an excess (10 μM) of desipramine. Specific binding was the difference between the two values.

Data analysis

Data were analyzed by the EBDA and LIGAND computer software programs (Elsevier-Biosoft, UK). Final estimates of IC_{50} and nH values were computed by the EBDA program. Baseline values for the individual drugs were established by computer analysis, using the baseline drugs as a guide. The LIGAND program provided final parameter estimates for the affinity of the radioligand (Kd) by iterative nonlinear curve-fitting and evaluation of one- or two-component binding models. The graphs displayed in this report were produced by the computer software program PRISM, using a one- or two-site competition analysis curve.

SPECT and MRI brain imaging

For the synthesis of the ^{99m}Tc complex, the unprotected ligand was dissolved in water and added to ^{99m}Tc-Glucoheptonate (Du Pont Merck Pharma, Billerica, MA) for ligand exchange and the compound purified by high-performance liquid chromatography (HPLC). One adult female rhesus monkey weighing 6.5 kg was fasted overnight and lightly anesthetized with ketamine (15 mg/kg). An indwelling catheter was placed in the saphenous vein and the animal maintained with Ringer's lactate solution at a rate of 10 ml/kg/h. Anesthesia for

the imaging session was obtained with an intramuscular injection of ketamine (15 mg/kg) and xylazine (1.5 mg/kg). During the study, one-half the ketamine/xylazine dose was given each hour for the duration of the study. The ^{99m}Tc compound (^{99m}Tc-technepine) was administered intravenously (7.5 mCi) via the catheter and was flushed with 1 ml saline. Images were acquired using a digital ASPECT system (Digital Scintigraphics, Inc., Boston, MA). The procedure for reconstruction of the data is described elsewhere (Holman et al., 1994). Images were acquired in a sequence of 10-min counts for 3 h. Coronal, sagittal, and rotating three-dimensional displays were calculated from these data. To develop neuroanatomical correlates, magnetic resonance imaging (MRI) scans on the same monkey were acquired on a 1.5T Sigma Scanner (GE Medical Systems, Milwaukee, WI) at 1-mm slice thickness.

RESULTS AND DISCUSSION

Dopamine transporter affinity

Four rhenium-chelated phenyltropanes (Fig. 1) were investigated. In order to determine whether structure–activity relationships previously developed for phenyltropane binding to the dopamine transporter extend to this novel series, the transporter binding affinities of the four rhenium analogues were determined. O-861R is a rhenium chelate analogue of WIN 35,428; O-862R is an analog of WIN 35,140 (2α-epimer of WIN 35,065-2, Madras et al., 1989a); O-863R is an analogue of dichloropane (Meltzer et al., 1993); O-864R is an analogue of difluoropine (Meltzer et al., 1994). The affinity and transporter selectivity of the rhenium chelate compounds were tested in radio-receptor assays as described in Methods. Each drug inhibited [^{3}H]WIN 35,428 binding to the dopamine transporter in a concentration-dependent manner (Fig. 2A). Interestingly, the binding isotherm for O-861R and O-863R plateaued at 2–10% above the baseline level established for cocaine and cocaine congeners. A similar plateau has previously been observed for noncocaine congeners that are potent dopamine transport inhibitors, such as GBR 12909 or mazindol (Madras et al., 1989b). The binding isotherms for O-861R were relatively steep, and the pseudo-Hill coefficient was approximately 1. LIGAND analysis of the data revealed that the data modeled to a single site on the dopamine transporter, in contrast with WIN 35,428, which consistently modeled to two sites (Madras et al., 1989b).

Within the series, two rhenium analogues, O-861R and O-862R, retained binding affinities similar to those of the parent compounds, WIN 35,428 and WIN 35,140. O-861R was the most potent drug at the dopamine transporter, binding with twice the affinity (IC_{50}: 5.99 ± 0.81 nM; n = 7) as the parent compound WIN 35,428 (Table I). The high-affinity binding was not surprising, since compounds with bulky groups on the tropane nitrogen retain affinity for the dopamine transporter (Meltzer et al., in press). Stereochemical binding was preserved in this series. Stereochemical constraint requires phenyltropanes to be in the 2β-configuration for high-affinity binding. In this regard, WIN 35,428, a 2β- is 260 times more potent than WIN 35,140, a 2α-epimer (Madras et al., 1989a,b). Likewise, O-861R, the corresponding 2β-rhenium chelate is 500 times more potent than O-862R, the 2α-epimer corresponding to WIN 35,140, a nondalozenated phenyltropane.

Two other rhenium chelate analogues displayed reduced binding potency compared with the parent compounds. O-863R, the rhenium chelate analogue of di-

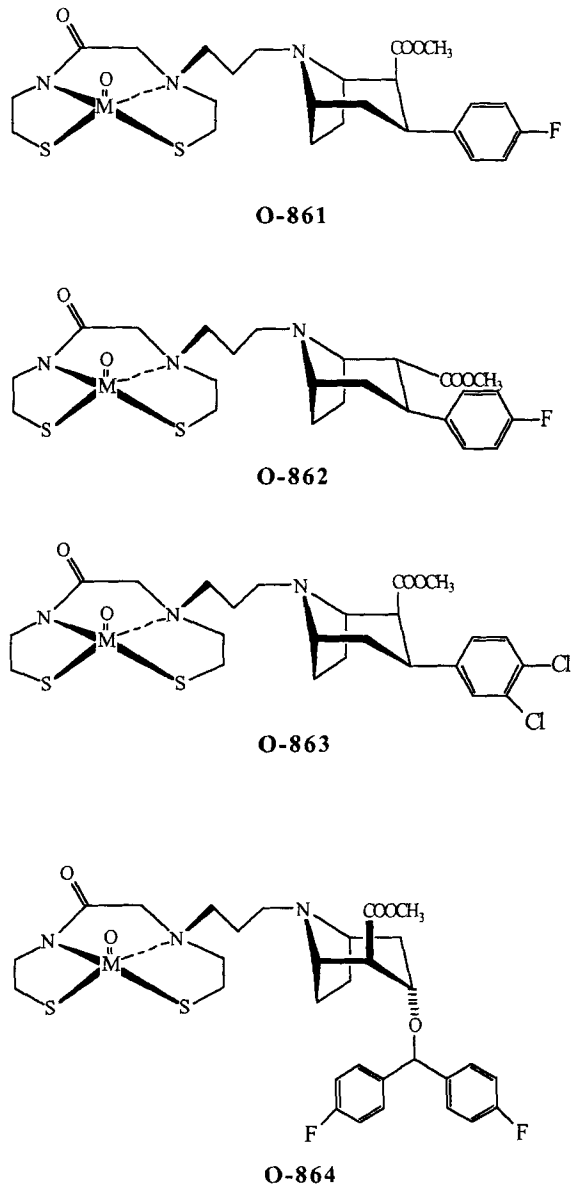
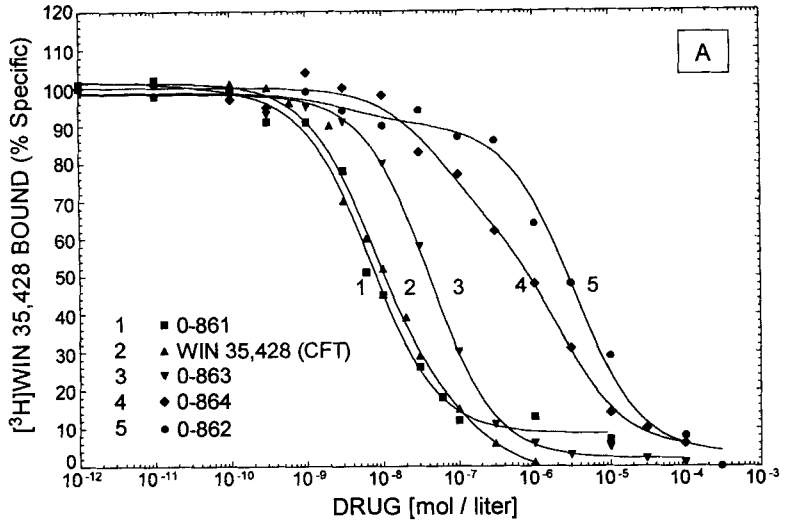
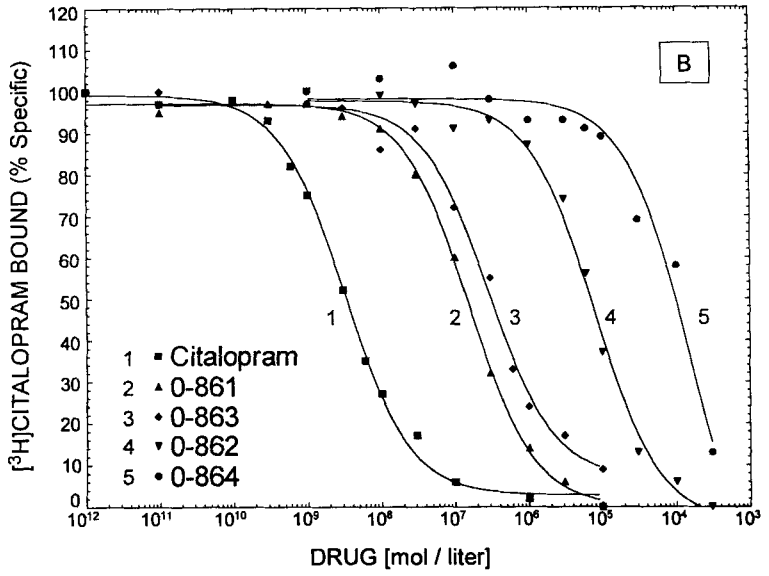


Fig. 1. Structures and labeling of compounds referred to in Table I and in the text.


A
[3H]WIN 35,428 BOUND (% Specific)
DRUG [mol / liter]
1 ■ 0-861
2 ▲ WIN 35,428 (CFT)
3 ▼ 0-863
4 ◆ 0-864
5 ● 0-862


B
[3H]CITALOPRAM BOUND (% Specific)
DRUG [mol / liter]
1 ■ Citalopram
2 ▲ 0-861
3 ◆ 0-863
4 ▼ 0-862
5 ● 0-864

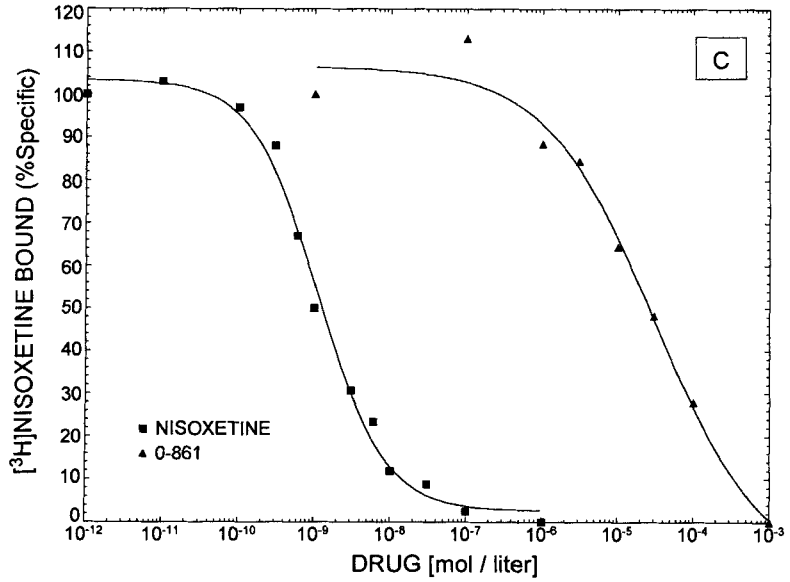

C
[3H]NISOXETINE BOUND (%Specific)
DRUG [mol / liter]
■ NISOXETINE
▲ 0-861

Figure 2

TABLE I. Affinity of novel compounds at the dopamine (DA), serotonin (5-HT), and norepinephrine (NE) transporters[1]

Drug	DA IC_{50} (nM)	5-HT IC_{50} (nM)	NE IC_{50} (nM)	DA/5-HT Selectivity ratio	DA/NE Selectivity ratio
WIN 35,428 (CFT)	11.0 ± 1.0	160 ± 20	748*	15	68
0-861 R	5.99 ± 0.81	124 ± 17	40,300 ± 10,300	21	6,730
WIN 35,140	2,900 ± 310	—	—	—	—
0-862 R	2,960 ± 157	5,020 ± 1,880	—	1.7	—
Dichloropane	1.09 ± 0.02	2.47 ± 0.14	—	2.3	—
0-863 R	37.2 ± 3.4	264 ± 16	—	7.1	—
Difluoropine	10.9 ± 1.2	3,530 ± 78	184 ± 26	324	17
0-864 R	616 ± 88	55,200 ± 20,000	—	90	—

[1]Various concentrations of drugs were incubated with [^{3}H]WIN 35,428 (DA), [^{3}H] citalopram (5-HT), and [^{3}H]nisoxetine (NE) and striatum homogenates (DA, 5-HT) or thalamus homogenates (NE), as described under Methods. Each drug was tested in two to seven independent experiments each conducted in triplicate. Results are expressed as means ± SEM. The affinity of the novel compound is given below the parent compound.
*n = 1.

chloropane (dichloro analogue of WIN 35,428), was 34 times less potent than dichloropane, one of the most potent drugs at the dopamine transporter. Difluoropine is a high-affinity benztropine analogue of WIN 35,428 that, unlike (*R*)-cocaine or (*R*)-WIN 35,428, is more potent in the (*S*) configuration and possesses a 3α-substituent on the tropane (Meltzer et al., 1994). The rhenium chelate analogue of difluoropine, O-864R, was 60-fold less potent than difluoropine (Table I). Taken together, these data indicate that structure–activity relationships developed for the WIN series of compounds do not fully correspond to those of the rhenium chelate analogues. The presence of a large N-substituent modifies the association of tropanes with the dopamine transporter. The affinity of the compounds for the serotonin transporter, labeled with [^{3}H]citalopram, was determined in parallel assays using the same brain tissue (Table I, Fig. 2B). Based on IC_{50} values, O-861R was more selective for the dopamine over the serotonin transporter than WIN 35,428. The most selective compound was O-864R, which was essentially inactive at the serotonin transporter and the least selective compounds were O-862R and O-863R, analogues of WIN 35,140 and dichloropane, respectively. A general trend for preservation of dopamine–serotonin transporter selectivity ratios of the parent phenyltropane compounds was observed in this small series of compounds.

As the norepinephrine transporter density is extremely low in the striatum (Gehlert et al., 1995), the affinity of compounds for this transporter is relevant principally for extrastriatal regions of brain or peripheral tissues (e.g., heart) that contain higher levels of this transporter. In pilot studies, we measured the affinity of O-861R for the norepinephrine transporter in thalamus, a brain region that yields substantial amounts of tissue, a relatively high specific–nonspecific binding ratio and a pharmacological binding profile of [^{3}H]nisoxetine that corresponds to that of the norepinephrine carrier (Madras et al., in preparation). The affinity of O-861R for the norepinephrine transporter was very low (IC_{50}: 40,300 ± 10,300 nM, n = 4), and the selectivity ratio (dopamine–norepinephrine transporter) was 6,700 (Table I, Figure 2C). These data highlight O-861R as one of the most selective drugs for the dopamine over the norepinephrine transporter. The rhenium chelates of tropanes may be useful for clarifying the topography of the binding domains of the three monoamine transporters. Most importantly, these data suggest that membrane-bound receptors or transporters are accessible to drugs complexed to a bulky metal chelate.

Based on previous experience, relatively high affinity and high selectivity for the dopamine over the serotonin (and the norepinephrine transporter) are vital in vitro criteria for initial identification of a potential brain imaging agent for the dopamine transporter. O-861R displayed high affinity (low nanomolar) and high selectivity for the dopamine over the serotonin and norepinephrine transporters. In comparison to WIN 35,428, which has favorable properties for PET imaging, O-861R is more potent and more selective for the dopamine transporter. Consequently, O-861R was selected for labeling with (^{99m}Tc) to observe whether

Fig. 2. Binding of rhenium compounds to the dopamine, serotonin, and norepinephrine transporter. **A:** Inhibition of [^{3}H]WIN 35,428 ([^{3}H]CFT) binding (0.3 nM) to the dopamine transporter by novel compounds. Caudate-putamen membranes of cynomolgus monkey brain were incubated with various concentrations of the test compound and the radioligand. The competition curve for WIN 35,428 is used as a reference standard for each brain preparation. Each curve is representative of two to seven independent experiments, each conducted in triplicate. **B:** Inhibition of [^{3}H]citalopram binding to the serotonin transporter in caudate-putamen membranes of cynomolgus monkey brain by novel compounds. The competition curve for citalopram is used as a reference standard for each brain preparation. Each curve is representative of two to three independent experiments, each conducted in triplicate. **C:** Inhibition of [^{3}H]nisoxetine binding to the norepinephrine transporter in thalamus membranes of cynomolgus monkey brain by nisoxetine and O-861R. Each curve is representative of four independent experiments, each conducted in triplicate.

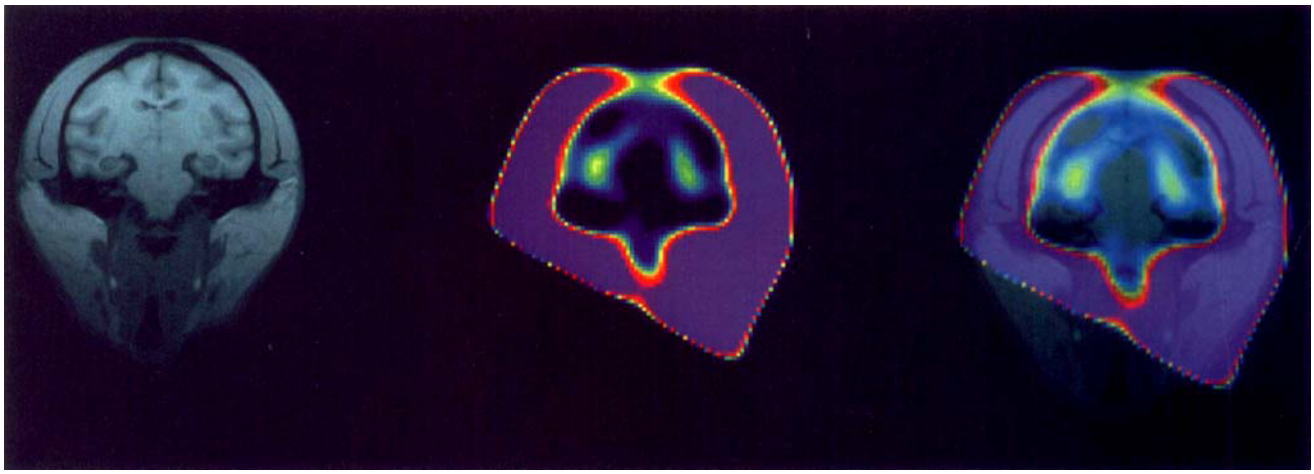


Fig. 3. Co-registered SPECT and MRI coronal sections of rhesus monkey brain, processed 1–1.5 h after injection of the radioactive probe. The putamen is the principal region highlighted and its location is identified by the MRI scan.

it crosses the blood-brain barrier and accumulates in the striatum.

The reliance on rhenium (Re) as a model for technetium is based on the following considerations. N_2S_2 complexes are equally stable for Re and Tc. Rhenium forms square pyramidal complexes with N_2S_2 ligands which are very similar to those formed by Tc (Francesconi et al., 1993). Consequently, it is often used in place of ^{99m}Tc in studies that do not require the presence of a radiolabel. X-ray and NMR data show that the structures of Tc and Re are similar and NMR shifts do not differ markedly from one to the other (Francesconi et al., 1993). Most importantly, the biological and log *P* values of Tc and Re chelates have been shown to be similar (O'Neil et al., 1994). This allows the nonradioactive rhenium to be used as a model for the radioactive technetium in biological assays.

SPECT imaging with O-861

SPECT imaging with the ^{99m}Tc analogue of O-861R was conducted in a female rhesus monkey (*Macaca mulatta*) as described in Methods. Within minutes of I.V. administration, technepine accumulated uniquely in the striatum and radioactivity was detectable in this brain region for as long as 3 h (Fig. 3). Co-registration of the SPECT images obtained in the coronal plane with coronal images generated by MRI confirmed the location of the radiolabeled probe within the striatum at several anterior-to-posterior planes. Further experiments are needed to affirm selectivity of the labeling at the dopamine transporter.

SPECT imaging agents that target the dopamine transporter are highly useful probes for monitoring the transporter and associated dopamine neurons. Applications for imaging the dopamine transporter in brain have expanded rapidly in recent years after radiolabeled WIN 35,428 and its analogues were identified as selective probes for the dopamine transporter. In addition to Parkinson's, Tourette's, and Lesch-Nyhan disease (Frost et al., 1993; Hantraye et al., 1992; Innis et al., 1993; Kaufman and Madras, 1991; Madras et al., 1990a; Seibyl et al., 1995; Wong et al., 1993, 1995), the transporter, labeled by phenyltropanes, may be a window on the age-related decline in dopamine and dopamine neurons (Kaufman and Madras, 1993; van Dyke et al., 1995). In substance abusers, the dopamine transporter in striatum is elevated in cocaine abusers (Malison et al., 1995) and is decreased markedly in alcohol abusers, but only in the nonviolent population (Tiihonen et al., 1995). Furthermore, imaging the transporter may also accelerate the development of long-acting cocaine medications (Madras et al., 1994).

O-861R is the first rhenium probe that binds with high affinity to the dopamine transporter and the ^{99m}technetium-labeled analogue is the first reported compound to cross the blood-brain barrier and accumulate in a selective target, the striatum. The brain entry of technepine was particularly exciting, as several attempts at developing technetium-labeled probes for specific targets in brain have not been successful (Lever et al., 1994; Del Rosario et al., 1994; Ballinger et al., 1989). Technetium is advantageous because ^{11}C or ^{123}I must be produced in a cyclotron whereas ^{99m}Tc ($t_{\frac{1}{2}} = 6$h) can be generated routinely in a laboratory from 99M. The evidence that technepine enters the brain suggests the feasibility of developing other technetium-labeled drugs targeted to brain transporters and to receptors.

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REFERENCES

Ballinger, J.R., Gulenchyne, K.Y., and Hassan, M.N. (1989) Tc-99m spiperone dithiocarbamate: A potential radiopharmaceutical for dopamine receptor imaging with SPECT. Appl. Rad. Isot., 40:547–549.

Boja, J.W., Patel, A., Carroll, F.I., Rahman, M.A., Philip, A., Lewin, A.H., and Kuhar, M.J. (1991) [^{125}I]RTI-55: A potent ligand for dopamine transporters. Eur. J. Pharmacol., 194:133–134.

Brownell, A-L, Elmaleh, D.E., Meltzer, P.C., Shoup, T.M., Brownell, G.L., Fischman, A.J., and Madras, B.K. (in press) Cocaine congeners as PET imaging probes for dopamine terminals in normal and MPTP induced Parkinsonism in nonhuman primate brain. J. Nucl. Med.

Canfield, D.R., Spealman, R.D., and Madras, B.K. (1989) Regional binding of the high affinity cocaine analog, [^{3}H]CFT, in monkey brain. Soc. Neurosci. Abs., 15:252.

Canfield, D.R., Spealman, R.D., Kaufman, M.J., and Madras, B.K. (1990) Autoradiographic localization of cocaine receptors by [^{3}H]CFT in monkey brain. Synapse, 5:189–195.

Carroll, F.I., Abraham, P., Lewin, A.H., Parham, K.A., Boja, J.W., and Kuhar, M. (1992) Isopropyl and phenyl esters of 3 beta-(4-substituted phenyl)tropan-2 beta-carboxylic acids. Potent and selective compounds for the dopamine transporter. J. Med. Chem., 35:2497–2500.

Clarke, R.L., Daum, S.J., Gambino, A.J., Aceto, M.D., Pearl, J., Levitt, M., Cumiskey, W.R., and Bogado, E.F. (1973) Compounds affecting the central nervous system. 4. 3β-Phenyltropane-2-carboxylic esters and analogs. J. Med. Chem., 16:1260–1267.

Del Rosario, R.B., Jung, Y.W., Baidoo, K.E., Lever, S.Z., and Wieland, D.M. (1994) Synthesis and in vivo evaluation of a $^{99m/99}$Tc-DADT-benzovesamicol: A potential marker for cholinergic neurons. Nucl. Med. Biol., 21:197–203.

Fischman, A.J., Babich, J.W., Elmaleh, D.R., Barrow, S.A., Meltzer, P., Hanson, R.N., and Madras, B.K. (submitted) SPECT imaging of dopamine transporter sites in normal and MPTP treated rhesus monkeys.

Francesconi, L.C., Graczyk, G., Wehrli, S., Shaikh, S.N., McClinton, D., Liu, S., Zibieta, J., and Kung, H.F. (1993) Synthesis and characterization of neutral MVO (M = Tc, Re) amine-thiol complexes containing a pendant phenylpiperidine group. Inorg. Chem., 32: 3114–3124.

Frost, J.J., Rosier, A.J., Reich, S.G., Smith, J.S., Ehlers, M.D., Snyder, S.H., Ravert, H.T., and Dannals, R.F. (1993) Positron emission tomographic imaging of the dopamine transporter with ^{11}C-WIN 35,428 reveals marked declines in mild Parkinson's disease. Ann. Neurol., 34:423–431.

Gehlert, D.R., Schober, D.A., and Gackenheimer, S.L. (1995) Comparison of (*R*)-[^{3}H]Tomoxetine and (*R*/*S*)-[^{3}H]Nisoxetine binding in rat brain. J. Neurochem., 64:2792–2800.

Hantraye, P., Brownell, A.-L., Elmaleh, D., Spealman, R.D., Wullner, U., Brownell, G.L., Madras, B.K., and Isacson, O. (1992) Dopamine fiber detection by ^{11}C-CFT and PET in a primate model of Parkinsonism. Neuro Reports, 3:265–268.

Heikkila, R.E., Cabbat, F.S., Manzino, L., and Duvoisin, R.C. (1979) Rotational behavior induced by cocaine analogs in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra: Dependence upon dopamine uptake inhibition. J. Pharmacol. Exp. Ther., 211: 189–194.

Holman, B.L., Carvahlo, P.A., Mendelson, J., Teoh, S.K., Nardin, R., Hallgring, E., Hebben, N., Johnson, K.A. (1994) Brain perfusion is abnormal in cocaine-dependent polydrug users: a study using technetium-99^{m}-HMPAO and ASPECT. J. Nucl. Med., 31:1206–1210.

Innis, R., Baldwin, R., Sybirska, E., Zea, Y., Laruelle, M., Al-Tikriti, M., Charney, D., Zoghbi, S., Smith, E., Wisniewski, G., Hoffer, P., Wang, S., Milius, R., and Neumeyer, J. (1991) Single photon emission computed tomography imaging of monoamine reuptake sites in primate brain with [^{123}I]CIT. Eur. J. Pharmacol., 200:369–370.

Kaufman, M.J., and Madras, B.K. (1991) Severe depletion of cocaine recognition sites associated with the dopamine transporter in Parkinson's diseased striatum. Synapse, 9:43–49.

Kaufman, M.J., and Madras, B.K. (1992) Distribution of cocaine recognition sites in monkey brain. II. Ex vivo autoradiography with [^{3}H]CFT and [^{125}I]RTI-55. Synapse, 12:99–111.

Kaufman, M.J., and Madras, B.K. (1993) [^{3}H]CFT ([^{3}H]WIN 35,428) accumulation in dopamine regions of monkey brain: Comparison of a mature and an aged monkey. Brain Res., 611:322–328.

Kaufman, M.J., Spealman, R.D., and Madras, B.K. (1991) Distribution of cocaine recognition sites in monkey brain. I. In vitro autoradiography with [^{3}H]CFT. Synapse, 9:177–187.

Kuhar, M.J., Sanchez-Roa, P.M., Wong, D.F., Dannals, R.F., Grigoriadis, D.E., Lew, R., and Milberger, M. (1990) Dopamine transporter: Biochemistry, Pharmacology and Imaging. Eur. Neurol., 30:15–20.

Lever, S.Z., Baidoo, K.E., Mahmood, A., Matsumura, K., Scheffel, U., and Wagner, H.N. (1994) Novel technetium ligands with affinity for the muscarinic cholinergic receptor. Nucl. Med. Biol., 21:157–164.

Madras, B.K. (1994) ^{11}C-WIN 35,428 for detecting dopamine depletion in mild Parkinson's disease. Ann. Neurol. Abs., 35:376–377.

Madras, B.K., Fahey, M.A., Bergman, J., Canfield, D.R., and Spealman, R.D. (1989a) Effects of cocaine and related drugs in nonhuman primates. I. [^{3}H]Cocaine binding sites in caudate-putamen. J. Pharmacol. Exp. Ther., 251:131–141.

Madras, B.K., Spealman, R.D., Fahey, M.A., Neumeyer, J.L., Saha, J.K., and Milius, R.A. (1989b) Cocaine receptors labeled by [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane. Mol. Pharmacol., 36: 518–524.

Madras, B.K., Fahey, M.A., and Kaufman, M.J. (1990a) [^{3}H]CFT and [^{3}H]Lu19-005: Markers for cocaine receptors/dopamine nerve terminals in Parkinson's disease. Soc. Neurosci. Abs., 16:14.

Madras, B.K., Kamien, J.B., Fahey, M., Canfield, D., Milius, R.A., Saha, J.K., Neumeyer, J.L., and Spealman, R.D. (1990b) N-modified fluorophenyltropane analogs of cocaine with high affinity for [^{3}H]cocaine receptors. Pharmacol. Biochem. Behav., 35:949–953.

Madras, B.K., Fahey, M.A., Kaufman, M.J., Spealman, R.D., Schumacher, J., Isacson, O., Brownell, A.-L., Brownell, G.L., and Elmaleh, D.R. (1991) Cocaine receptor probes in nonhuman and human brain: In vitro characterization and in vivo imaging. Soc. Neurosci. Abs., 17:190.

Madras, B.K. (1992) Brain imaging of the dopamine transporter. NIDA Brain Imaging Conference, Keystone, Co.

Madras, B.K., Elmaleh, D.R., Meltzer, P.C., Liang, A.Y., Brownell, G.L., and Brownell, A.-L. (1994) Positron Emission Tomography of cocaine recognition sites on the dopamine transporter. NIDA Res. Monogr., 138:57–69.

Madras, B.K., Gracz, L.M., Kaufman, M.J., Meltzer, P.C., Elmaleh, D.E., and Fischman, A.J. (1995) A novel SPECT imaging agent detects total loss of the dopamine transporter in Parkinsonian monkeys. Soc. Neurosci. Abs., 21:1983.

Malison, R.T., Best, S., Wallace, E.A., van Dyck, C.H., Zoghbi, S.S., Zea-Ponce, Y., Baldwin, R.M., Laruelle, M., Seibyl, J., McCance-Katz, E., Price, L.H., Kosten, T.R., and Innis, R.B. (1995) SPECT imaging of dopamine transporters in cocaine dependent and healthy control subjects with [^{123}I]β-CIT. J. Nucl. Med. Abs., 36:31P.

Meltzer, P.C., Liang, A.Y., Brownell, A.-L., Elmaleh, D.R., and Madras, B.K. (1993) Substituted 3-phenyltropane analogs of cocaine: Synthesis, inhibition of binding at cocaine recognition sites and positron emission tomography (PET) imaging. J. Med. Chem., 36:855–862.

Meltzer, P.C., Liang, A.Y., and Madras, B.K. (1994) The discovery of an unusually selective and novel cocaine analog: Difluoropine. Synthesis and inhibition of binding at cocaine recognition sites. J. Med. Chem. 37:2001–2010.

Meltzer, P.C., Liang, A.Y., and Madras, B.K. (in press) 2-Carbomethoxy-3-(diarylmethoxy)-1 α*H*,5α*H*-tropane analogs: Synthesis and inhibition of binding at the dopamine transporter and comparison with piperazines of the GBR series. J. Med. Chem.

Morris, E.D., Babich, J.W., Alpert, N.M., Bonab, A.A., Livni, E., Weise, S., Hus, H., Christian, B.T., Madras, B.K., and Fischman, A.J. (submitted) Quantification of dopamine transporter density in monkeys by dynamic PET imaging of multiple injections of ^{11}C-CFT: A pilot study. Synapse.

O'Neil, J.P., Carlson, K.E., Anderson, C.J., Welch, M.J., and Katzenellenbogen, J.A. (1994) Progestin radiopharmaceuticals labeled with technetium and rhenium: Synthesis, binding affinity, and in vivo distribution of a new progestin N_2S_2–metal conjugate. Bioconjugate Chem., 5:182–193.

Schoemaker, H., Pimoule, C., Arbilla, S., Scatton, B., Javoy-Agid, F., and Langer, S.Z. (1985) Sodium dependent [^{3}H]cocaine binding associated with dopamine uptake sites in the rat striatum and human putamen decrease after dopaminergic denervation and in Parkinson's disease. Naunyn-Schmiedebergs Arch. Pharmacol., 329:227.

Seeman, P. (1993) Receptor tables. Vol. 2: Drug Dissociation Constants for Neuroreceptors and Transporters. SZ Research, Toronto, Canada.

Seibyl, J.P., Marek, K.L., Quinlan, D., Sheff, K., Zoghbi, S., Zea-Ponce,

Y., Baldwin, R.M., Fussell, B., Smith, E.O., Charney, D.S., Hoffer, P.B., and Innis, R.B. (1995) Decreased single-photon emission computed tomographic [^{123}I]β-CIT striatal uptake correlates with symptom severity in Parkinson's disease. Ann. Neurol., 38:589–598.

Singer, H.S., Hahn, I.-H., and Moran, T.H. (1991) Abnormal dopamine uptake sites in postmortem striatum from patients with Tourette's syndrome. Ann. Neurol., 30:558–562.

Tiihonen, J., Kuikka, J., Bergström, K., Hakola, P., Karhu, J., Olli-Pekka, R., and Föhr, J. (1995) Altered striatal dopamine re-uptake site densities in habitually violent and non-violent alcoholics. Nature Med., 1:654–657.

van Dyke, C.H., Seibyl, J.P., Malison, R.T., Laruelle, M., Wallace, E., Zoghbi, S.S., Zea-Ponce, Y., Baldwin, R.M., Charney, D.S., Hoffer, P.B., and Innis, R.B. (1995) Age-related decline in striatal dopamine transporter binding with iodine-123-β-CIT SPECT. J. Nucl. Med., 36:1175–1181.

Wong, D.F., Yung, B., Dannals, R.F., Shaya, E.K., Ravert, H.T., Chen, C.A., Chan, B., Folio, T., Scheffel, U., Ricaurte, G.A., Neumeyer, J.L., Wagner, H.N., Jr., and Kuhar, M.J. (1993) In vivo imaging of baboon and human dopamine transporters by positron emission tomography using [^{11}C]WIN 35,428. Synapse, 15:130–142.

Wong, D.F., Harris, J., Singer, H., Naudi, S., Yokoi, F., Marenco, S., Evans, A., Rousset, O., Ma, Y., Yaster, M., Hong, C., Ravert, H., Dannals, R.F., Breese, G., Kuhar, M.J., and Gjedde, A. (1995) Dopamine receptor transporter imaging in neuropsychiatric developmental disorders. J. Cereb. Blood Flow Metab., 15:S97.