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Short communication

[^{3}H]β-CIT: a radioligand for dopamine transporters in rat brain tissue

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Abstract

[^{3}H]2-β-carbomethoxy-3-β-[4′-iodophenyl]tropane (β-CIT) was prepared and evaluated. With rat forebrain tissue, [^{3}H]β-CIT showed high affinity for dopamine transporters (DAT), with selectivity for DAT over norepinephrine transporters, but not serotonin transporters, as well as DAT-stereoselectivity with β-CIT, amphetamine and methylphenidate. Affinity and selectivity for 53 compounds assayed with [^{3}H]β-CIT and standard DAT radioligand [^{3}H]GBR-12935 were highly correlated ($r > 0.95$). [^{3}H]β-CIT is proposed as a useful, high-affinity DAT radioprobe.

Keywords: Autoradiography; [^{3}H]β-CIT; Dopamine; Monoamine; Transporter; Tropane

1. Introduction

Radioligands with high affinity or selectivity for dopamine transporters (DAT) include tritiated mazindol (Javitch et al., 1984), GBR-12935 (Andersen, 1987), phencyclidine analog *N*-(1-[1-benzo[b]thien-2-ylcyclohexyl])piperidine (BTCP) (Vignon et al., 1988) and phenyltropane congeners, including radioligands suitable for clinical neuroimaging (Milius et al., 1991; Chally et al., 1996; Neumeyer et al., 1994; La Garza et al., 1999). Phenyltropanes include [^{3}H]CFT (Madras et al., 1989) and [^{125}I]*R*-2β-carbomethoxy-3β-(4-iodophenyl)tropane (β-CIT, RTI-55) (Boja et al., 1991; Innis et al., 1991). Since [^{3}H]β-CIT is not available, we prepared and characterized it, finding close pharmacological similarity to the standard DAT radioligand [^{3}H]GBR-12935, and a very high signal-to-noise ratio in radiotransporter binding and autoradiographic experiments.

2. Materials and methods

2.1. Materials

Ten phenyltropanes were prepared (Neumeyer et al., 1994) at Research Biochemicals International (RBI, Natick MA): β-CIT, its precursor (*nor*-β-CIT), enantiomer (1S)-β-CIT, 3,4-diiodophenyl (CIIT), 4-fluorophenyl-(CFT) and 4-chlorophenyl congeners (CCIT), as well as *N*-2-fluoroethyl-CIT (FE-CIT), *N*-3-fluropropyl-CIT (FP-CIT), 2β-carboisopropoxy-CIT (CIT-IP), and *N*-3-fluoropropyl-2β-carboisopropoxy (FP-CIT-IP) derivatives. [*N*-^{3}H-methyl]β-CIT (87 Ci/mmol) was prepared from *nor*-β-CIT at New England Nuclear (NEN; Boston, MA). Other NEN radioligands included [propylene-2,3-^{3}H]GBR-12935 (13 Ci/mmol, $K_d = 1.0$ nM), [phenyl-6′-^{3}H](−)-paroxetine (20 Ci/mmol, $K_d = 150$ pM), and [*N*-methyl-^{3}H](±)-nisoxetine (85 Ci/mmol; $K_d = 800$ pM). Test agents were from RBI or Sigma (St. Louis, MO) or donated by: Celgene (Warren, NJ; methylphenidate–HCl isomers), Ferrosan (Copenhagen; [−]-paroxetine–HCl), Hoechst-Roussel (Sommerville, NJ; nomifensine-maleate), Eli Lilly (Indianapolis, IN; (±)-fluoxetine–HCl), Lundbeck

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(Copenhagen; [±]-citalopram–HBr), Novartis (Basle, Switzerland; mazindol), Pfizer (Groton, CT; [+]-sertraline–HCl), and Philips-Duphar (Amsterdam; fluvoxamine-maleate).

2.2. Cerebral tissue

Brain tissue from young adult (250 g), male Sprague–Dawley rats (Charles River Labs, Wilmington, MA; following federal guidelines and Institutional approval) included homogenates (in ice-cold, 50 mM Tris–HCl buffer, pH 7.4 with 150 mM NaCl) of caudate–putamen (CPu) or frontoparietal cerebral cortex, and cryostatic coronal sections (10 μm) through mid-striatum.

2.3. Radiotransporter assays

[^{3}H]β-CIT was incubated with striatal homogenates in assay buffer (50 mM Tris–citrate, pH 7.4 with 120 mM NaCl and 4 mM $MgCl_2$) at concentrations (C) of 25–4500 pM (optimized for routine assays at $C = 300$ pM, 60 min, 20°C), and compared with [^{3}H]GBR-12935 ($C = 400$ pM, 45 min, 4°C), with 1 μM GBR-12909 to define nonspecific binding (Kula and Baldessarini, 1990). [^{3}H](−)-Paroxetine ($C = 200$ pM, 60 min, 20°C; blank 2 μM fluoxetine) labeled 5-HT transporters (5-HTT) (Habert et al., 1985), and [^{3}H]nisoxetine ($C = 270$ pM, 180 min, 4°C; blank 2 μM desipramine) labeled norepinephrine transporters (NET) (Tejani-Butt, 1992) in cortical homogenates in 50 mM Tris–HCl (pH 7.4) with 5 mM KCl and 120 mM NaCl (300 mM for NET). Assays were terminated on ice, filtered (glass-fiber filters saturated with 0.3% [vols] polyethyleneimine), washed with excess ice-cold 150 mM saline, and counted in Polyfluor (Packard Instruments; Meriden, CT) in a LS spectrophotometer (Wallac-LKB; Gaithersburg, MD; 50% efficiency).

[^{3}H]β-CIT K_d was determined by Scatchard and kinetic analyses (1 μM GBR-12909 used for k_{off}, with $K_d = k_{off}/k_{on}$) (Kula and Baldessarini, 1990; Baldessarini et al., 1992). Concentration-inhibition functions were based on ≥2 independent analyses involving ≥6 concentrations (in triplicate) of each test agent, following screening at 1, 3 and 10 μM. Hill slopes and $IC_{50} \pm$ S.E., converted to K_i (nM) ($K_i = IC_{50}/[1 + (C/K_d)]$) were determined by computer-function-fitting (Baldessarini et al., 1992). K_i for 53 test agents with both DAT radioligands, and K_i selectivity-ratios (for 5-HT/DA and NE/DA radiotransporter assays) were compared by linear regression of their negative logarithms (pK_i or p[K_i-ratio]).

2.4. Autoradiography

Cryostatic coronal sections (10 μm) through mid-striatum were preincubated (60 min, 20°C) in DAT assay buffer, then 60 min in fresh buffer with 2 nM [^{3}H]β-CIT or 2 nM [^{3}H]GBR-12935 (with 1 μM *cis*-flupenthixol, to prevent labeling of piperazine sites), with nonspecific binding defined with 1 μM GBR-12909 with/without 1 μM citalopram. Slides were washed twice (5 min in ice-cold fresh buffer), dipped in ice-cold water, dried, exposed to tritium-sensitive film for 10 days with [^{3}H]standards, photodeveloped, and analyzed by computed densitometry, all detailed elsewhere (Tarazi et al., 1998).

3. Results

3.1. Binding of [^{3}H]β-CIT with rat striatal homogenates

Striatal binding of [^{3}H]β-CIT was linear vs. time (1–30 min, saturating by 45 min at 20°C), and linearly dependent on tissue-protein (to ±5-times standard assay conditions equivalent to 1.5 mg fresh striatum). Unlabeled β-CIT inhibited [^{3}H]β-CIT binding with striatal homogenates monophasically (slope function, 0.99). K_d of [^{3}H]β-CIT by Scatchard and kinetic analyses averaged 230 pM. At standard assay $C = 300$ pM [^{3}H]β-CIT, specific binding defined with 1 μM GBR-12909 averaged 92%.

3.2. Pharmacology of binding of [^{3}H]β-CIT compared with [^{3}H]GBR-12935

Potency (K_i, nM) of 53 compounds competing vs. [^{3}H]β-CIT for binding to presumptive DAT sites in striatal membranes, was compared with K_i vs. [^{3}H]GBR-12935 (Table 1). Hill slope functions with compounds with $K_i < 1$ μM averaged: 0.998 ± 0.036 with [^{3}H]β-CIT and 0.857 ± 0.025 with [^{3}H]GBR-12935.

Expected isomeric preference was found with *R*-over-*S*-β-CIT, (+)-over-(−)-amphetamine, and (+)-over-(−) methylphenidate with both radioligands. Phenyltropane affinities were similar with both radioligands, but CFT was 57-times less potent than β-CIT vs. [^{3}H]β-CIT, and preferred [^{3}H]GBR-12935 over [^{3}H]β-CIT by 5-fold. Similar K_i-rank-order was shown with both DAT radioligands by DAT active agents, with lower affinity for other comparison agents (Table 1).

Some agents selective for 5-HTT or NET (Table 1) had expected weak interactions with both DAT radioprobes (K_i all ≥1 μM with fluoxetine, desipramine, fluvoxamine, citalopram), but several antidepressants usually considered selective for 5-HTT or NET had some DAT affinity with both radioligands (sertraline, paroxetine, nisoxetine: $K_i = 14$–506 nM). Mazindol was NET-selective (51–114-times), but showed considerable DAT- as well as 5-HTT affinity (K_i 16–38 nM), and nomifensine favored NET-over-DAT by 16–25-fold.

Overall correlation of pK_i values for compounds tested with both DAT probes (Table 1) was very high ($r = 0.988$, slope = 0.992, $p < 0.0001$). Moreover, selectivity (pK_i-ratios) for 5-HT/DA and NE/DA transporters was similar with both DAT radioligands ($r = 0.993$, slope = 0.942 for DAT-over-5-HTT, and $r = 0.992$, slope = 0.971 for DAT-over-NET; both $p < 0.0001$).

Table 1
Affinity (K_i, nM ± S.E.) at monoamine transporters in rat brain tissue. The following compounds showed < 10% receptor binding activity at > 10,000 nM with both DA_T radioligands: atropine, benzoylnorecognine, *m*-benzoylecognine, *p*-benzoylnorecognine, bretylium, (−)-epinephrine, guanethidine, (−)-norepinephrine, octopamine, propylamine, serotonin, *m*-tyramine, *p*-tyramine

Test Compound	Dopamine		Serotonin	Norepinephrine
	[^{3}H]β-CIT	[^{3}H]GBR-12935	[^{3}H]Paroxetine	[^{3}H]Nisoxetine
Tropanes				
nor-β-CIT	0.64 ± 0.097	0.42 ± 0.06	0.062 ± 0.001	1.85 ± 0.21
CIIT	1.26 ± 0.04	0.96 ± 0.08	0.38 ± 0.03	50.8 ± 3.0
β-CIT	1.33 ± 0.15	0.96 ± 0.15	0.46 ± 0.06	2.80 ± 0.40
CIT-IP	1.85 ± 0.25	3.28 ± 0.22	20.8 ± 1.5	592 ± 50
CCIT	2.36 ± 0.17	1.75 ± 0.07	6.40 ± 0.32	17.5 ± 4.6
FE-CIT	7.19 ± 0.74	3.67 ± 0.43	0.86 ± 0.06	93.0 ± 17
FP-CIT	8.29 ± 0.53	3.53 ± 0.34	1.68 ± 0.13	63.0 ± 4.0
FP-CIT-IP	15.6 ± 1.7	8.83 ± 1.45	48.7 ± 8.4	≥ 10,000
CFT	76.0 ± 2.3	14.7 ± 2.9	181 ± 21	635 ± 110
p-OH–Cocaine	230 ± 13	170 ± 50	3600 ± 400	773 ± 68
Cocaine	400 ± 50	350 ± 67	1500 ± 200	1500 ± 250
m-OH–Cocaine	720 ± 200	470 ± 75	1500 ± 200	7000 ± 900
(1S)-β-CIT	> 10,000	> 10,000	558 ± 66	> 10,000
Dopamine transport blockers				
GBR-12909	0.15 ± 0.05	0.06 ± 0.02	52.8 ± 4.4	> 10,000
GBR-12935	1.59 ± 0.02	0.46 ± 0.05	1000 ± 150	1500 ± 250
Indatraline	1.77 ± 0.12	0.90 ± 0.09	0.12 ± 0.02	1.17 ± 0.16
BTCP	3.90 ± 0.70	5.60 ± 0.57	66.1 ± 4.7	53.2 ± 7.7
GBR-13069	4.00 ± 0.10	1.07 ± 0.15	160 ± 22	2000 ± 300
Amfonelic acid	18.7 ± 1.3	5.64 ± 0.9	> 10,000	> 10,000
(+)-Methylphenidate	125 ± 10	54.3 ± 6.2	> 10,000	126 ± 7.0
(±)-Methylphenidate	211 ± 23	82.9 ± 15.7	> 10,000	242 ± 15
(−)-Methylphenidate	1500 ± 200	451 ± 118	> 10,000	3000 ± 400
Benztropine	242 ± 22.0	52.6 ± 38.3	383 ± 24	1000 ± 200
GYKI-52895	378 ± 42	281 ± 35	> 10,000	> 10,000
Bupropion	840 ± 72	168 ± 21	> 10,000	≥ 10,000
(+)-Amphetamine	1000 ± 150	1000 ± 150	> 10,000	1000 ± 150
(−)-Amphetamine	> 10,000	> 10,000	> 10,000	> 10,000
Serotonin or norepinephrine transporter ligands				
Sertraline	20.0 ± 2.9	13.8 ± 3.9	0.16 ± 0.01	> 10,000
Mazindol	37.6 ± 91.4	16.9 ± 9.7	36.1 ± 9.7	0.33 ± 0.08
Nomifensine	76.5 ± 6.7	48.9 ± 22	2600 ± 350	3.11 ± 0.38
Paroxetine	355 ± 52	506 ± 66	0.90 ± 0.30	324 ± 47.0
Nisoxetine	505 ± 50	286 ± 25	158 ± 29	0.460 ± 0.20
Fluoxetine	1700 ± 250	1100 ± 200	3.55 ± 0.29	6000 ± 800
Desipramine	> 10,000	7000	228 ± 20	0.061 ± 0.041
Fluvoxamine	> 10,000	> 10,000	2.77 ± 0.17	5000 ± 600
Citalopram	> 10,000	> 10,000	0.820 ± 0.030	> 10,000
Miscellaneous compounds				
Chlorpheniramine	3000 ± 400	867 ± 126	45.3 ± 4.8	1500 ± 250
Dopamine	> 10,000	2500 ± 300	> 10,000	> 10,000
R(−)-Apomorphine	> 10,000	5000 ± 600	> 10,000	> 10,000
Tranylcypromine	> 10,000	≥ 10,000	> 10,000	3000 ± 400

3.3. Autoradiography

[^{3}H]β-CIT autoradiography yielded well-defined signals-over-background. Labeling was highly selective for CPu and nucleus accumbens septi (NAc), with ≥ 11% as much specific radiographic density in other regions, including frontal cortex. Nonspecific binding (with 1 μM GBR-12909) accounted for only 8.0%, and 11.1% of total [^{3}H]β-CIT binding in CPu and NAc, where specific DAT binding (± S.E.M., $N = 5$) ranked: 249 ± 12.6 and 154 ± 3.5 fmol/mg tissue, respectively. The weak remaining signal with [^{3}H]β-CIT + GBR-12909 in striatum fell virtually to background with 1 μM citalopram included.

In contrast to [^{3}H]β-CIT, alternate rat brain sections evaluated under matched conditions with [^{3}H]GBR-12935 (with *cis*-flupenthixol to mask piperazine binding sites)

yielded lower proportions of specific to total radioligand binding (70.0% in CPu, 59.1% in NAc), and 1 μM citalopram reduced background only slightly, consistent with the low affinity of GBR-12935 vs. 5-HTT ligand [^{3}H]paroxetine with cerebral cortical homogenates (K_i = 1180 nM; Table 1).

4. Discussion

Other than [^{3}H]β-CIT, the only other commercially available tritiated-phenyltropane for labeling DAT is [^{3}H]CFT, studied with primate brain tissue (Madras et al., 1989). Unlabeled β-CIT showed 57- and 15-fold higher affinity than CFT vs. [^{3}H]β-CIT and [^{3}H]GBR-12935 (Table 1). [^{3}H]β-CIT was selective for DAT sites in autoradiographs of corpus striatum: 90% of total binding was displaced by the dissimilar but very potent DAT ligand, GBR-12909 (Table 1). The little [^{3}H]β-CIT bound in extrastriatal areas was virtually completely displaced by the potent, very highly 5-HTT-selective agent citalopram. These findings suggest only minor cross-reaction of [^{3}H]β-CIT to 5-HTT in striatum. Nevertheless, [^{125}I]β-CIT (RTI-55) can label 5-HTT in serotonin-rich sites, including raphe nuclei (Fujita et al., 1991).

There was very close pharmacological similarity of [^{3}H]β-CIT and standard DAT-radioligand [^{3}H]GBR-12935, with expected stereoselectivity for several enantiomeric-pairs and similar potency-rankings for 53 test-agents with or without DAT-selectivity. With both radioligands, there were also high correlations of pK_i values and of selectivity (pK_i-ratios) for DAT over both 5-HTT and NET. Binding of [^{3}H]β-CIT was monophasic, though the radioiodinated-β-CIT ([^{125}I]RTI-55) detected two binding sites (Boja et al., 1991).

The present results support the utility of [^{3}H]β-CIT as a radioligand for the DAT, with high DAT-affinity, very high proportion of specific binding in rat striatum, and close pharmacological similarity to standard DAT radioligand [^{3}H]GBR-12935. [^{3}H]β-CIT should be useful for radiotransporter assays and autoradiographic analysis of DAT-rich tissues.

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