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Short communication

RBI-257: A highly potent dopamine D_4 receptor-selective ligand

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Abstract

RBI-257 (1-[4-iodobenzyl]-4-[*N*-(3-isopropoxy-2-pyridinyl)-*N*-methyl]-aminopiperidine), the *p*-iodobenzyl analog of U-101,958 (1-benzyl-4-[*N*-(3-isopropoxy-2-pyridinyl)-*N*-methyl]-aminopiperidine) had a lower dissociation constant ($K_i = 0.3$ vs. 2.7 nM) and higher selectivity than U-101,958 at dopamine D_4 receptors, over dopamine D_2 and D_3 receptors in transfected cell membranes and D_2-like sites in rat forebrain. Dopamine D_4 receptor affinity of iodo-isomers of RBI-257 ranked: *para* > *meta* > *ortho*. RBI-257 had much lower affinity at D_1 and D_5 dopamine receptors in transfected cells, as well as dopamine D_1-like receptors, α_1, α_2 or $\beta_{1,2}$ adrenoceptors, $\sigma_{1,2}$ receptors and 5-HT_{1A} or 5-HT_{2A} receptors, and transporters for dopamine, norepinephrine or serotonin in rat forebrain tissue. RBI-257 may be a useful probe or radioligand for brain dopamine D_4 receptors. © 1997 Elsevier Science B.V.

Keywords: Dopamine; Dopamine D_4 receptor; RBI-257; U-101,958

1. Introduction

Discovery of mRNA for a dopamine D_4 receptor in mammalian brain and of partial selectivity of the superior antipsychotic agent clozapine for dopamine D_4 receptors over dopamine D_2 receptors (Van Tol et al., 1991), as well as continuing uncertainty about the location of dopamine D_4 receptor protein in brain tissue (Tarazi et al., 1997), have stimulated a search for agents with high affinity and selectivity for this receptor (Baldessarini and Tarazi, 1996; Kebabian et al., 1997). Among new agents with such selectivity, the Upjohn compound U-101,958 (1-benzyl-4-[*N*-(3-isopropoxy-2-pyridinyl)-*N*-methyl]-aminopiperidine) is a promising lead (Schlachter et al., 1995). We now report on the *p*-iodobenzyl analog of this compound (1-[4-iodobenzyl]-4-[*N*-(3-isopropoxy-2-pyridinyl)-*N*-methyl]-aminopiperidine; RBI-257). RBI-257 showed very high affinity for dopamine D_4 receptors expressed in genetically transfected cells, similar to that of other new dopamine D_4 receptor-selective ligands (Kulagowski et al., 1996; Patel et al., 1996; Zorn et al., 1996; Kebabian et al., 1997), as well as high selectivity over the major dopamine receptor types ($D_{1/2/3/5}$) and several other potential sites of interaction in brain tissue.

2. Materials and methods

2.1. Tissue preparation

Materials for radioreceptor assays included membranes of cells genetically transfected to express specific dopamine receptor types, or homogenates prepared from brain tissue of rat (250 g; Charles River, Wilmington, MA, USA) or frozen guinea pig (minus cerebellum; Rockland, Gilbertsville, PA, USA), as specified in Table 1. Receptor preparations selectively expressing types 1, 2L, 3, 4.2, or 5 dopamine receptors were supplied by Research Biochemicals International (Natick, MA, USA), and prepared by recommendations provided by the supplier.

2.2. Chemicals and drugs

RBI-257 and its iodo positional isomers, U-101,958, (+)-butaclamol-HCl, (−)-eticlopride-HCl and phentolamine mesylate were provided by RBI. Other drugs were obtained as follows: *cis*-flupenthixol-di-HCl (gift of Lund-

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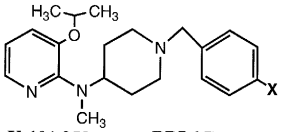
Table 1
Dissociation constants (K_i) for dopamine D_4 ligand candidates at various receptor types



U-101,958 (X = H) **RBI-257** (X = I)

Target	Tissue	Ligand	Blank	RBI-257		U-101,958	
				$K_i \pm$ S.E.	Selectivity	$K_i \pm$ S.E.	Selectivity
D_4	CHO cells	0.5 nM [^{3}H]spiperone	10 μM haloperidol	0.33 ± 0.05	1.0	2.67 ± 0.11	1.0
D_{2L}	Sf9 cells	0.16 nM [^{3}H]spiperone	1.0 μM haloperidol	568 ± 97	1721	1980 ± 144	742
D_3	CCL1.3 cells	0.09 nM [^{3}H]spiperone	0.1 μM (−)-eticlopride	145 ± 32	439	552 ± 101	207
D_2-like	rat striatum	0.07 nM [^{3}H]nemonapride	1.0 μM (+)-butaclamol	441 ± 41	1336	803 ± 229	301
D_1	Sf9 cells	1.0 nM [^{3}H]SCH-23390	1.0 μM (+)-butaclamol	2830 ± 245	8576	> 10 000	> 10 000
D_5	Sf9 cells	1.0 nM [^{3}H]SCH-23390	10 μM (+)-butaclamol	> 10 000	> 10 000	> 10 000	> 10 000
D_1-like	rat striatum	0.3 nM [^{3}H]SCH-23390	0.3 μM *cis*-flupenthixol	≥ 10 000	> 10 000	> 10 000	> 10 000
α_1	rat forebrain	0.2 nM [^{3}H]prazosin	2.0 μM phentolamine	ca. 10 000	> 10 000	ca. 10 000	> 10 000
α_2	rat forebrain	1.0 nM [^{3}H]MK-912	10 μM phentolamine	ca. 10 000	> 10 000	ca. 10 000	> 10 000
$\beta_{1,2}$	rat cerebral cortex	0.5 nM [^{3}H]dihydroalprenolol	10 μM propranolol	> 20 000	> 10 000	> 30 000	> 10 000
$\sigma_{1,2}$	guinea-pig brain	1.85 nM [^{3}H]ditolylguanidine	10 μM haloperidol	82.1 ± 1.7	249	75.2 ± 23.4	28.2
5-HT_{2A}	rat forebrain	0.4 nM [^{3}H]ketanserin	10 μM cinanserin	273 ± 25	827	1076 ± 147	403
5-HT_{1A}	rat forebrain	1.0 nM [^{3}H]8-OH-DPAT	10 μM serotonin	> 10 000	> 10 000	> 10 000	> 10 000
5-HT_T	rat cerebral cortex	0.2 nM [^{3}H]paroxetine	10 μM fluoxetine	207 ± 22	627	> 30 000	> 10 000
DA_T	rat striatum	0.4 nM [^{3}H]GBR-12935	30 μM methylphenidate	> 10 000	> 10 000	> 10 000	> 10 000
NE_T	rat cerebral cortex	0.8 nM [^{3}H]nisoxetine	10 μM desipramine	> 100 000	> 100 000	> 100 000	> 100 000

Data are dissociation constants ($K_i \pm$ S.E.). 5-HT_T, DA_T and NE_T are serotonin, dopamine and norepinephrine transporters. Selectivity = ratio of K_i at each comparison site to K_i at dopamine D_4 receptors. In addition, the positional isomers of RBI-257 were tested at dopamine D_4 receptors: for the *meta*-iodo compound (RBI-285), $K_i = 0.91 \pm 0.07$ nM; for the *ortho*-iodo congener (RBI-284), $K_i = 12.4 \pm 0.60$ nM.

beck, Copenhagen, Denmark), cinanserin-HCl (gift of E.R. Squibb, Princeton, NJ, USA); desipramine-HCl, haloperidol, leupeptin, methylphenidate-HCl, phenylmethylsulfonylfluoride, polyethylenimine and 5-hydroxytryptamine-HCl (serotonin, Sigma, St. Louis, MO, USA); (±)-fluoxetine-HCl (gift of Eli Lilly, Indianapolis, IN, USA); propranolol-HCl (gift of Ayerst, New York, NY, USA). Other chemicals were of the highest available chemical grade. [^{3}H]Radioligands (specific activity in Ci/mmol) were: spiperone (98; Amersham, Arlington Heights, IL, USA); and (±)-SCH-23390 (7-chloro-8-hydroxy-1-phenyl-3-methyl-2,3,4,5-tetrahydro-1-*H*-benzazepine; 85), (±)-nemonapride (YM-09151-2; 85), prazosin (81); MK-912 ([2*S-trans*]-1,3,4,5′,6,6′,7,12*b*-octahydro-[1′,3′-dimethylspiro[2*H*-benzofuro-(2,3*a*)-quinolizine-2,4′-(1′*H*)-pyrimidin]-2′[3′*H*]-one; 77); dihydroalprenolol (120); ditolylguanidine (35); ketanserin (81), (±)-8-OH-*N*,*N*-di-*n*-propylaminotetralin (8-OH-DPAT; 154), GBR-12935 (1[2-(diphenylmethoxy)ethyl]-4-[3-phenylpropyl]-piperazine; 13), nisoxetine (86) and paroxetine (20 Ci/mmol) were from New England Nuclear (Boston, MA, USA).

2.3. Assay procedures

Receptor and transporter affinities were determined with tissues, radioligands and blanking agents defined in Table 1. Affinity of test agents at dopamine D_1-like and D_2-like receptors in brain tissue were tested with homogenates of rat corpus striatum (Baldessarini et al., 1994). Other assays also followed previously reported methods: affinity to α_1- and α_2-adrenoceptors was tested with homogenates of rat forebrain (Baldessarini et al., 1994); $\beta_{1,2}$-adrenoceptors with homogenates of rat cerebral cortex (Bylund and Snyder, 1975); and 5-HT_{1A} (Taylor et al., 1988) and 5-HT_{2A} receptors with homogenates of rat forebrain (Leysen et al., 1982); and $\sigma_{1,2}$ sites were assayed in homogenates of guinea-pig brain (Weber et al., 1986). Affinity to membrane transporter proteins for dopamine, norepinephrine and serotonin were tested with homogenates of rat corpus

striatum or cerebral cortex (Neumeyer et al., 1996). For dopamine D_1, D_{2L}, D_3 and D_5 receptor assays with cell membranes, assay buffer was Tris–HCl (50 mM, pH 7.4, with 150 mM NaCl). For assays of dopamine D_4 receptors, assay buffer was modified to include (mM), NaCl (120), KCl (5), EDTA (5), $CaCl_2$ (1.5), and protease inhibitors phenylmethylsulfonylfluoride (1) and leupeptin (1 μg/ml), and also used to homogenize and wash the frozen $D_{4.2}$ cell pellets before their use.

Cell membranes (5–10 μg protein/tube) or homogenates (100–200 μg protein/tube) were incubated with test agents (RBI-257 and U-101,958) in 6–8 concentrations in 2–4 replicates and radioligand (at concentration C, of separately determined dissociation constant, K_d) with and without a blank agent (Table 1), at optimal volume (0.4–1.0 ml), temperature (20–30°C) and time (20–120 min), as described in the references cited. Following incubation, samples were collected on glass fiber filter sheets (No. 32; Schleicher&Schuell, Keene, NH, USA) saturated with 0.3% (vol%) polyethylenimine, in a Brandel Cell Harvester (Gaithersburg, MD, USA) under vacuum. Bound radioactivity was then counted in minivials containing 3.5 ml Polyfluor (Packard Instruments, Downers Grove, IL, USA) in liquid scintillation counter (Wallac, Gaithersburg, MD, USA) at approx. 50% efficiency. Values for half-maximally effective inhibitory concentration, as $IC_{50} \pm$ S.E. (nM) were determined by computer-fitting the data (percent of control specific binding vs. concentration of test agent) with the ALLFIT program and converted to $K_i \pm$ S.E., as $K_i = IC_{50}/[1 + K_d/C]$ (Baldessarini et al., 1994).

3. Results

The affinity ($1/K_i$) of three positional isomers of the iodinated compounds for dopamine D_4 receptors ranked: *para-* > *meta-* > *ortho*-iodo, and the *para*-iodo compound (RBI-257) was evaluated further (Table 1). Compared to its nonhalogenated congener, U-101,958, RBI-257 had a more than eight-fold lower dissociation constant ($K_i = 0.3$ vs. 2.7 nM) and more than two-fold greater selectivity for D_4 over D_{2L} (1700 vs. 740 times) or D_3 dopamine receptors (439 vs. 207 times). Both agents (and the positional isomers of RBI-257 (not shown)) also had low affinity to dopamine D_2-like receptor sites in rat striatum (mainly D_{2L}; Baldessarini and Tarazi, 1996). Interactions of both compounds at dopamine D_1 and D_5 receptors in membranes of transfected cells, as well as at dopamine D_1-like receptor sites in rat brain tissue were negligible (K_i all 2800–10 000 nM).

Additional evidence of the selectivity of both RBI-257 and U-101,958 for dopamine D_4 receptors included their negligible affinity at α- or β-adrenoceptors, serotonin 5-HT_{1A} receptors and the transporters for dopamine (DA_T) or norepinephrine (NE_T; K_i all > 10 000 nM; Table 1). RBI-257 had low affinity at serotonin transporters (5-HT_T) and 5-HT_{2A} receptors (K_i = 207 and 273 nM) but this was greater than the affinity of U-101,958 at both sites (K_i = > 30 000 and 1076 nM, respectively). Finally, both dopamine D_4 receptor ligands showed some affinity for sigma sites, although RBI-257 was 8.9-times more selective for dopamine D_4 vs. sigma sites (249-fold) than was U-101,958 (28-fold; Table 1).

4. Discussion

These results add to growing evidence that compounds of very high affinity and selectivity for dopamine D_4 receptors can be devised (Kebabian et al., 1997). The evident enhancement of affinity and selectivity for dopamine D_4 receptors by adding iodine in the novel compound RBI-257 may reflect a gain in lipid solubility commonly associated with halogenation. The beneficial effect of iodination also encourages developing a radioiodinated derivative of RBI-257 for use in experimental and perhaps clinical neuroradiological studies. In view of current uncertainty of the functional significance of the dopamine D_4 receptors and their low cerebral abundance (Baldessarini and Tarazi, 1996; Tarazi et al., 1997), highly selective ligands like RBI-257 may help in defining their anatomical distribution and pharmacological properties.

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