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Short communication

The novel antagonist, S33084, and GR218,231 interact selectively with cloned and native, rat dopamine D_3 receptors as compared with native, rat dopamine D_2 receptors

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Abstract

The novel benzopyranopyrrole, S33084 ((3a*R*,9b*S*)-*N*[4-(8-cyano-1,3a,4,9b-tetrahydro-3*H*-benzopyrano[3,4-*c*]pyrrole-2-yl)-butyl] (4-phenyl)benzamide)), and the aminotetralin derivative, GR218,231 (2(*R*,*S*)-(di-*n*-propylamino)-6-(4-methoxyphenylsulfonylmethyl)-1,2,3,4-tetrahydro naphthalene), displayed high affinity at cloned, rat dopamine D_3 receptors (pK_is of 8.72 and 8.67, respectively), as well as dopamine D_3 receptors in rat olfactory tubercle (8.62 and 8.94, respectively). In contrast, they showed low affinities at striatal dopamine D_2 receptors (6.82 and 6.64, respectively). Unlike S33084 and GR218,231, the arylpiperazine, L741,626 (4-(4-chlorophenyl)-1-(1*H*-indol-3-ylmethyl)piperidin-4-ol), showed lower affinity for cloned (6.46) and native (6.92) dopamine D_3 receptors than for striatal dopamine D_2 receptors (7.52). S33084, GR218,231 and L741,626 should prove useful tools for exploration of the functional roles of dopamine D_3 vs. dopamine D_2 receptors.

Keywords: Dopamine D_3 receptor; Limbic system

1. Introduction

Although transgenic and antisense strategies have provided important insights into functional roles of dopamine D_3 compared with dopamine D_2 receptors (Baik et al., 1995; Accili et al., 1996; Tepper et al., 1997; Ekman et al., 1998), there remains a need for selective dopamine D_3 receptor antagonists as experimental tools and — potentially — therapeutic agents (Levant, 1997; Wustrow and Wise, 1997). While several, preferential antagonists at dopamine D_3 receptors have been described, some, such as the benzamide, nafadotride, and the aminoindane, U99194 ({5,6-dimethoxy-indan-2-yl) dipropylamine}), show only limited ($\leq$ 10-fold) selectivity versus dopamine D_2 (and other) receptors (Sautel et al., 1995; Audinot et al., 1998; Haadsma-Svensson and Svensson, 1998). Although more selective, the arylpipazine, GR103,691({4′-acetyl-*N*-{4-[(2-methoxy-phenyl)-piperazin-1-yl]-butyl}-biphenyl-4-carboxamide), displays significant affinity for serotonin 5-HT_{1A} receptors and α_1-adrenoceptors and only poor bioavailability (Murray et al., 1995; Audinot et al., 1998). Further, while the benzofurane, S14297 ((+)-[7-(*N*,*N*-dipropylamino)-5,6,7,8-tetrahydro-naphto(2,3b)dihydro,2,3-furane]), manifests marked selectivity and satisfactory pharmacokinetics, it retains weak partial agonist activity at cloned human dopamine D_3 and D_2 receptors (Millan et al., 1995; Cussac et al., 1999a; Newman-Tancredi et al., 1999). In contrast to the above, the aminotetralin GR218,231 (2(*R*,*S*)-(di-*n*-propylamino)-6-(4-methoxyphenylsulfonylimethyl)-1,2,3,4- tetrahydro naphthalene) (Murray et al., 1996), and the recently identified benzopyranopyrrole, S33084 ((3a*R*,9b*S*)-*N*[4-(8-cyano-1,3a,4,9b-tetrahydro-3*H*-benzopyrano[3,4-*c*]pyrrole-2-yl)-butyl](4-phenyl)benzamide)) (Cussac et al., 1999b; Dubuffet et al., 1999; Millan et al., submitted), unite the desired characteristics of high potency, pronounced selectivity and functional activity in vivo. Further, S33084 shows $>$ 200-fold selectivity for human dopamine D_3 receptors vs. all other ($>$ 40) sites examined (Cussac et al., 1999b; Millan et al., submitted).

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However, in common with other dopamine D_3 receptors antagonists, the affinities of GR218,231 and S33084 have, to date, only been determined at heterologously expressed, cloned, human dopamine D_3 and D_2 receptors (Murray et al., 1996; Cussac et al., 1999b; Dubuffet et al., 1999). To underpin the potential utility of GR218231 and S33084 as pharmacological tools, it is important to establish that they similarly act as potent ligands of rat dopamine D_3 receptors. To this end, we determined the affinities of S33084 and GR218,231 at cloned and native rat dopamine D_3 as compared with native dopamine D_2 receptors (Gackenheimer et al., 1995; Bancroft et al., 1998). The affinities of S33084 and GR218,231 were compared with those of the preferential dopamine D_2 receptor antagonist, L741,626 (4-(4-chlorophenyl)-1-(1*H*-indol-3-ylmethyl)-piperidin-4-ol) (Kulagowski et al., 1996; Levant, 1997; Bancroft et al., 1998).

2. Materials and methods

2.1. Affinities at cloned rat dopamine D_3 and striatal rat dopamine D_2 receptors

Drug affinities at cloned rat dopamine D_3 receptors expressed in Chinese hamster ovary cells and at rat (striatal) dopamine D_2 receptors were determined employing standard protocols described previously (Millan et al., 1995). Competition binding experiments were carried out with [^{3}H]spiperone (0.2 nM). Non-specific binding was defined with (+)-butaclamol (10 μM) and raclopride (10 μM) for cloned rat dopamine D_3 and striatal rat dopamine D_2 sites, respectively.

2.2. Affinities at rat olfactory tubercle-localised dopamine D_3 receptors

Olfactory tubercles (1/75, w/v) were homogenized using a polytron in a buffer containing Tris 50 mM (pH 7.5), EDTA 5 mM and GTPγS 100 μM, centrifuged in the same buffer for 20 min at 40,000 × *g* at 4°C. Membranes were resuspended in this buffer and incubated for 15 min at 37°C followed by a centrifugation for 20 min at 40,000 × *g* at 4°C. For competition binding experiments, membranes were incubated with ligands in the presence of [^{3}H]7-OHDPAT (2 nM) at room temperature for 60 min in a buffer containing Tris 50 mM (pH 7.5), NaCl 120 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 5 mM and GTPγS 100 μM. Non-specific binding was defined with raclopride (10 μM). Experiments were terminated by rapid filtration through Whatman GF/B filters (pre-treated with 0.3 % polyethyleneimine) using a Brandel cell harvester. Radioactivity retained on the filters was determined by liquid scintillation counting.

2.3. Data analysis

Isotherms were analysed by non-linear regression, using the program 'PRISM' (Graphpad Software, San Diego, CA) to yield IC_{50} values. Inhibition constants (K_i values) were derived from IC_{50} values according to the Cheng–Prusoff equation.

2.4. Drug structures, sources and salts

[^{3}H]spiperone (104 Ci/mmol) and [^{3}H]7-OHDPAT (7-hydroxy-2-(di-*n*-propylamino)-tetralin) (150 Ci/mmol) were purchased from Amersham (Les Ulis, France). S33084 was synthesized by G. Lavielle (Servier) and GR218,231 by J.-L. Peglion (Servier). L741,626 was purchased from Tocris Cookson, Bristol, UK. PD 128,907 ((+)-(4a*R*,10b*R*)-3,4,4a,10b-tetrahydro-4-propyl-2*H*, 5*H*-[1]benzopyrano-[4,3-b]-1,4-oxazin-9-ol)) was purchased from Research Biochemicals International (Natick, MA, USA).

3. Results

At cloned, rat dopamine D_3 receptors, both GR218,231 and S33084 potently displaced [^{3}H]spiperone (Table 1). In distinction, L741,626 showed only modest affinity for these sites. Both 7-OH-DPAT and PD128,907 were potent ligands at cloned, rat dopamine D_3 receptors (Table 1). In rat olfactory tubercle, S33084 and GR218,231 potently displaced [^{3}H]7-OH-DPAT in the presence of GTPγS (100 μM), (Fig. 1 and Table 1). On the other hand, the affinity of L741,626 was modest. The affinities of 7-OH-DPAT and PD128,907 were high at these sites. S33084 and GR218,231 weakly displaced [^{3}H]spiperone binding at striatal dopamine D_2 receptors (Fig.1). In contrast, L741,626 showed marked affinity for striatal dopamine D_2 receptors.

Table 1
Binding affinities of dopaminergic ligands at cloned and native rat dopamine D_3 and D_2 receptors
Data are means ± S.E.M. of ≥ 3 determinations for native rat dopamine D_2 and D_3 receptors, and mean ± range ($n = 2$) for cloned dopamine D_3 rat receptors.

Ligand	Binding affinity (pK_i)		
	Cloned rat dopamine D_3 receptors	Native rat dopamine D_3 receptors	Native rat dopamine D_2 receptors
S33084	8.72 ± 0.19	8.62 ± 0.05	6.82 ± 0.05
GR218,231	8.67 ± 0.07	8.94 ± 0.11	6.64 ± 0.10
L741,626	6.46 ± 0.08	6.92 ± 0.07	7.52 ± 0.01
7-OH-DPAT	8.51 ± 0.07	9.01 ± 0.01	6.67 ± 0.06
PD128,907	8.61 ± 0.13	8.48 ± 0.11	7.28 ± 0.07

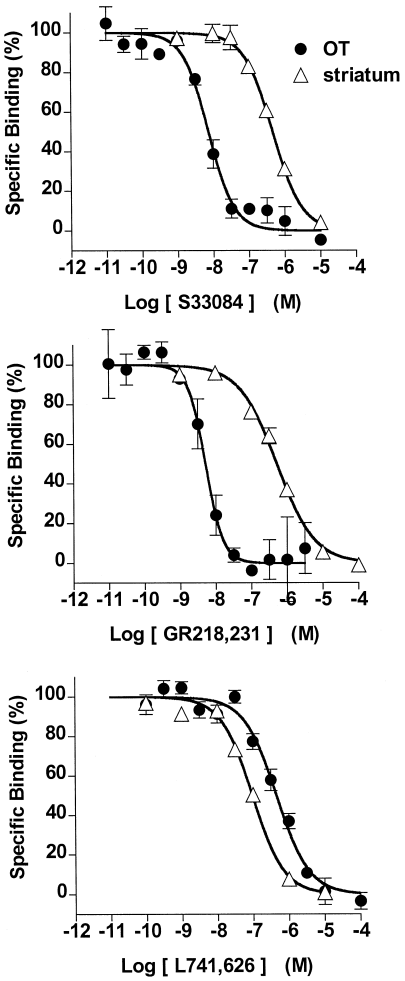

Fig. 1. Interaction of S33084, GR218,231 and L741,626 at olfactory tubercle-localised dopamine D_3 receptors and striatal dopamine D_2 receptors. Data points shown are means of triplicate determinations from a representative experiment repeated on at least three occasions. For olfactory tubercle, all isotherms yielded Pseudo-Hill (nH) coefficients did not differ significantly ($P > 0.05$) from unity: S33084, nH = 1.09 ± 0.12 ($n = 4$); GR218,231, nH = 1.09 ± 0.24 ($n = 4$) and L741,626, nH = 1.02 ± 0.23 ($n = 3$). For pK_i values, see Table 1.

The affinities of PD128,907 and 7-OH-DPAT at striatal dopamine D_2 sites were low (Table 1).

4. Discussion

In analogy to PD128,907 and other preferential dopamine D_3 receptor agonists employed for radiolabelling of dopamine D_3 sites, 7-OH-DPAT possesses significant affinity for the ''high-affinity'' state of dopamine D_2 receptors (Gackenheimer et al, 1995; Gonzales and Sibley, 1995; Bancroft et al., 1998). The GTP analogue, GTPγS, in decoupling dopamine D_2 receptors from the associated G-protein, suppresses this high-affinity state: that is, it permits the isolation of a GTP-resistant component of [^{3}H]7-OH-DPAT binding corresponding to dopamine D_3 receptors (Gackenheimer et al., 1995; Bancroft et al., 1998; Missale et al., 1998; Newman-Tancredi et al., 1999). Under these conditions, [^{3}H]7-OH-DPAT labels a homogeneous population of dopamine D_3 sites in the olfactory tubercle (Fig. 1), a structure in which dopamine D_3 receptors occur in a relatively high density (Gackenheimer et al., 1995; Bancroft et al., 1998). Correspondingly, in analogy to cloned, rat dopamine D_3 receptors, both 7-OH-DPAT itself and PD128,907 displayed markedly higher affinity in displacing [^{3}H]7-OH-DPAT from dopamine D_3 receptors in the olfactory tubercle than in displacing [^{3}H]spiperone binding to dopamine D_2 receptors in the striatum, which contains only a low density of dopamine D_3 sites (see Levant, 1997).

In analogy to 7-OH-DPAT and PD128,907, both S33084 and GR218,231 potently displaced [^{3}H]7-OH-DPAT binding in the olfactory tubercle with affinities close to those obtained at cloned, rat dopamine D_3 receptors, and considerably higher than their affinities for dopamine D_2 sites in striatum, a structure poor in dopamine D_3 sites (Gackenheimer et al., 1995) (Fig. 1). The affinities of S33084 and GR218,231 determined herein are similar to those observed at cloned, human dopamine D_3 vs. D_2 receptors: for S33084, 9.4 vs. 7.3 and for GR218,231, 8.9 vs. 6.9. In contrast, L741,626 showed a mild preference for striatal dopamine D_2 receptors compared with cloned, rat and olfactory tubercle-localised dopamine D_3 sites. These data resemble those obtained for cloned, human dopamine D_3 vs. D_2 receptors: 7.0 vs. 8.2, respectively. Thus, in distinction to L741,626, both S33084 and GR218,231 are preferential ligands at native, cerebral populations of dopamine D_3 versus dopamine D_2 receptors in the rat (Kulagowski et al., 1996; Murray et al., 1996; Cussac et al., 1999b; Millan et al., submitted). Although the present study did not address the issue of intrinsic activity, parallel studies have demonstrated competitive antagonist properties of GR218,231 and S33084 at human dopamine D_3 receptors and, at higher concentrations, at human dopamine D_2 receptors in vitro (Cussac et al., 1999a,b). Both S33084 and GR218,231 also behave as antagonists in in vivo models of dopamine D_3 and D_2 receptor-mediated activity in rodents (Cussac et al., 1999b; Millan et al., submitted).

[^{3}H]7-OH-DPAT can label σ_1 sites in striatum, and possesses modest affinity for serotonin 5-HT_{1A} receptors (Schoemaker, 1993; Millan et al., 1995). However, S33084 and GR218,231 possess negligible affinity ($pK_i < 6$) for these sites (Millan, et al., unpublished observation). Further, their competition binding isotherms against [^{3}H]7-OH-DPAT binding in the olfactory tubercle were monophasic, suggesting interaction with a single, high affinity site. These observations, and the similar affinities of S33084 and GR218,231 at cloned dopamine D_3 receptors as compared with olfactory tubercle-localised rat dopamine D_3 sites, exclude a role of σ_1 and/or serotonin

5-HT_{1A} sites in the displacement by GR218,231 and S33084 of [^{3}H]7-OH-DPAT binding in rat olfactory tubercle.

In conclusion, while L741,626 shows a mild preference for native, rat dopamine D_2 vs. D_3 sites, S33084 and GR218,231 are potent and preferential ligands of cloned and native, rat dopamine D_3 vs. D_2 receptors. Together with GR218,231 and L741,626, the novel benzopyranopyrrole, S33084, which displays >200-fold selectivity for human dopamine D_3 receptors versus all other (>40) sites evaluated (Cussac et al., 1999b; Millan et al., submitted), should provide an instructive pharmacological tool for exploration of the functional significance of dopamine D_3 receptors compared with dopamine D_2 receptors.

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