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The naphtosultam derivative RP 62203 (fananserin) has high affinity for the dopamine D_4 receptor

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Abstract

The dopamine D_4 receptor is a potential target for novel antipsychotic drugs. Most available compounds with affinity for the dopamine D_4 receptor also bind to dopamine D_2 receptors. This report describes the affinity of the 5-HT_{2A} receptor antagonist RP 62203 (fananserin) for the human dopamine D_4 receptor. Fananserin displaces [^{3}H]spiperone binding to recombinant human dopamine D_4 receptors with a K_i of 2.93 nM. This compares with an affinity (K_i) of 0.37 nM for the rat 5-HT_{2A} receptor and of 726 nM for the rat dopamine D_2 receptor. [^{3}H]Fananserin can be used to label the recombinant dopamine D_4 receptor expressed in Chinese hamster ovary cells with a K_D of 0.725 nM. Fananserin is, thus, the first compound to be reported that distinguishes between dopamine D_4 and D_2 receptors.

Keywords: RP 62203; Fananserin; Dopamine; Dopamine D_4 receptor; 5-HT_{2A} receptor; 5-HT (serotonin)

1. Introduction

It has been accepted for many years that antipsychotic drugs exert their effects by blocking dopamine receptors in the central nervous system. Since the original classification of dopamine receptors into D_1 and D_2 subtypes, three further subtypes have been identified by homology cloning strategies (Seeman and Van Tol, 1994). The dopamine D_4 receptor, cloned in 1991 (Van Tol et al., 1991), resembles both in structure and pharmacological properties the dopamine D_2 receptor. Most antipsychotics, with the exception of certain substituted benzamide drugs, such as remoxipride (Van Tol et al., 1991), bind with similar affinities to dopamine D_2 and D_4 receptor subtypes.

The dopamine D_4 receptor has, however, received much attention because of the observation (Van Tol et al., 1991) that the atypical antipsychotic clozapine has higher affinity for the dopamine D_4 receptor than for the dopamine D_2 receptor. Since clozapine has a somewhat unusual therapeutic profile, and is the only antipsychotic which does not occupy a high proportion of dopamine D_2 receptors at therapeutic doses (Farde et al., 1992; Seeman and Van Tol, 1994), much interest has been shown in the dopamine D_4 receptor as a potential target for clozapine-like antipsychotic drugs. Moreover, as dopamine D_4 receptors are not present in the striatum (Mrzljak et al., 1996), one might expect selective dopamine D_4 receptor antagonists to have fewer or no extrapyramidal effects than dopamine D_2 receptor antagonists.

However, the absence of molecules that bind selectively to dopamine D_4 receptors and not to dopamine D_2 receptors has hindered both understanding of the role of this receptor in brain function, and testing of the therapeutic interest of dopamine D_4 receptor antagonists. Five years after its identification, many doubts still surround the pertinence of this receptor for the mechanism of action of antipsychotic drugs (Roth et al., 1995; Reynolds, 1996).

A number of years ago, we described (Doble et al., 1992b) the pharmacology of the 5-HT_{2A} receptor antagonist RP 62203 (fananserin). In the present report, we provide evidence that fananserin also has high affinity for the human dopamine D_4 receptor and does not bind to the human dopamine D_2 receptor.

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2. Materials and methods

2.1. Materials

[^{3}H]Spiperone (specific activity: 98 Ci/mmol), [^{3}H]ketanserin (specific activity: 81 Ci/mmol) and [^{3}H]fananserin (specific activity: 56 Ci/mmol) were purchased from Amersham. Methysergide, fananserin, cinanserin, prazosin, sulpiride, ritanserin, risperidone, haloperidol, clozapine, olanzapine and seroquel were synthesised in the Chemistry Department at Rhône Poulenc Rorer.

2.2. Membrane preparation

The cerebral cortices and striata of male Sprague-Dawley rats were dissected out and homogenised separately in ice-cold Tris-HCl buffer (50 mM; pH 7.6). The homogenate was centrifuged for 10 min at 50 000 × g at 4°C and the supernatant discarded. The pellet was washed by re-suspension in 7 vols. of the same buffer and re-centrifugation. The final pellet was re-suspended in 5 vols. of Tris-HCl buffer, stored at −80°C and used for the 5-HT_{2A} receptor and dopamine D_2 receptor binding assays, respectively. Membranes containing recombinant human dopamine D_4 receptors ($hD_{4.2}$ variant) were obtained from transfected Chinese hamster ovary cells, provided by Receptor Biology (Baltimore, MD, USA).

Protein determinations were performed by the bicinchoninic acid method (Smith et al., 1985).

2.3. Binding assays

For the 5-HT_{2A} receptor binding assay, cortical membranes (0.15 mg ml^{-1}) were incubated for 15 min at 37°C in Tris-HCl buffer (50 mM; pH 7.6) containing [^{3}H]ketanserin (0.4 nM) and the compound of interest or methysergide (10^{-6} M) to define the non-specific binding. For the dopamine D_2 receptor binding assay, striatal membranes (0.05 mg ml^{-1}) were incubated for 45 min at 25°C in Tris-HCl buffer (50 mM; pH 7.6) containing NaCl (150 mM), cinanserin (10^{-6} M), prazosin (10^{-6} M), [^{3}H]spiperone (0.05 nM) and the compound of interest or sulpiride (10^{-6} M) to define the non-specific binding. Both of these binding assays were terminated by filtration across Whatman GF/B glass fibre filters using a Skatron cell harvester followed by two washes with 2.5 ml of ice-cold buffer.

Membranes from cells expressing recombinant human dopamine D_4 receptors (9 μg ml^{-1}) were incubated for 60 min at 25°C in Tris-HCl buffer (50 mM; pH 7.6) containing NaCl (120 mM), KCl (5 mM) $MgCl_2$ (5 mM), EDTA (1 mM), [^{3}H]spiperone (0.5 nM) or [^{3}H]fananserin (1 nM) and the compound of interest or haloperidol (10^{-6} M) to define the non-specific binding. These two binding assays were terminated by filtration across Wallac filtermat A glass fibre filters, pre-soaked with polyethylenimine 0.1% (4 h), using a Skatron cell harvester followed by two washes with 15 ml of ice-cold buffer.

In all cases, the radioactivity retained on the filters was determined by liquid scintillometry in 4 ml of Ready-Solv scintillant (Beckman).

2.4. Data analysis

Inhibition constants were derived from 7-point inhibition curves, each performed in triplicate (except for the dopamine D_4 receptor assay, which was performed in duplicate due to the expense of the material). IC_{50} values were calculated from curve-fitting to a simple Langmuir isotherm by computer-assisted non-linear regression analysis. The inhibition constant (K_i) was calculated from the IC_{50} value by the Cheng-Prusoff equation ($K_i = IC_{50}/(1 + ([L]/K_D))$). The Hill coefficients were calculated by non-linear regression analysis using a Langmuir isotherm in which the Hill coefficient was allowed to vary. In the case of saturation analysis, the dissociation equilibrium constant (K_D) and the binding capacity (B_{max}) were calculated from Scatchard transformations of 15-point saturation curves.

3. Results

3.1. Binding to dopamine D_4 receptors

Fananserin displaced [^{3}H]spiperone binding to human recombinant dopamine D_4 receptors in Chinese hamster ovary cell membranes with a K_i value of 2.93 nM (Table 1, Fig. 1a). Apart from haloperidol, all the other antipsychotics tested had lower affinity for fananserin at this receptor. The somewhat high value obtained for clozapine (106 nM) may be explained by the high salt concentration used in the assay (Van Tol et al., 1992). Hill coefficients of these inhibition curves were generally close to unity.

3.2. Binding to dopamine D_2 receptors

On the other hand, fananserin had very low affinity for the rat dopamine D_2 receptor in rat striatal membranes labeled with [^{3}H]spiperone ($K_i = 726$ nM), being less potent than any other antipsychotic tested (Table 1, Fig. 1b). The value obtained is similar to that previously published (Doble et al., 1992b). Hill coefficients of these inhibition curves were close to or somewhat higher than unity. Similarly, fananserin had little affinity for the human recombinant dopamine D_2 receptor expressed in Chinese hamster ovary cells ($K_i > 1000$ nM; data not shown). The selectivity of fananserin for dopamine D_4 over dopamine D_2 receptors was approximately 250, higher than for any other compound tested.

Table 1
Receptor affinities of fananserin and antipsychotic drugs

	D_4 receptor		D_2 receptor	5-HT_{2A} receptor	$K_{i\,[D2]}/K_{i\,[D4]}$
	Human recombinant receptor		Rat striatal membranes	Rat cortical membranes	
	[^{3}H]spiperone	[^{3}H]fananserin	[^{3}H]spiperone	[^{3}H]ketanserin	
Fananserin	$2.93 \pm 0.54 \times 10^{-9}$ (1.13)	$1.87 \pm 0.64 \times 10^{-9}$ (0.96)	$7.26 \pm 0.87 \times 10^{-7}$ (1.25)	$3.66 \pm 0.33 \times 10^{-10}$ (0.86)	248
Clozapine	$1.06 \pm 0.57 \times 10^{-7}$ (0.97)	$7.67 \pm 5.42 \times 10^{-8}$ (0.74)	$2.06 \pm 0.23 \times 10^{-7}$ (1.09)	$1.85 \pm 0.67 \times 10^{-8}$ (0.83)	1.94
Haloperidol	$2.17 \pm 0.09 \times 10^{-9}$ (1.01)	$3.33 \pm 0.74 \times 10^{-9}$ (0.77)	$1.85 \pm 0.48 \times 10^{-9}$ (0.89)	$5.50 \pm 1.78 \times 10^{-8}$ (0.82)	0.85
Olanzapine	$2.03 \pm 0.12 \times 10^{-8}$ (1.00)	$6.93 \pm 2.30 \times 10^{-8}$ (1.04)	$3.30 \pm 1.35 \times 10^{-8}$ (1.37)	$6.66 \pm 2.65 \times 10^{-9}$ (1.27)	1.63
Risperidone	$6.80 \pm 4.21 \times 10^{-9}$ (1.12)	$1.21 \pm 0.52 \times 10^{-8}$ (0.72)	$3.37 \pm 0.38 \times 10^{-9}$ (0.96)	$6.66 \pm 1.76 \times 10^{-10}$ (0.98)	0.50
Ritanserin	$2.19 \pm 0.41 \times 10^{-7}$ (1.36)	$5.47 \pm 2.03 \times 10^{-7}$ (0.84)	$1.12 \pm 0.17 \times 10^{-7}$ (1.06)	$1.73 \pm 1.39 \times 10^{-9}$ (0.96)	0.51
Seroquel	$> 1 \times 10^{-5}$	N.D.	$3.88 \pm 0.85 \times 10^{-7}$ (1.12)	$3.72 \pm 1.33 \times 10^{-7}$ (0.80)	< 0.04

Affinities of fananserin and several antipsychotic drugs for dopamine D_4 and D_2 receptors and 5-HT_{2A} receptors in membrane preparations are given in M. Data are expressed as K_i values, and represent the mean ± S.E.M. of three independent determinations. The numbers in parentheses represent the Hill coefficients of the inhibition curves. For the calculation of the K_i ratios, the $K_{i\,[D4]}$ values obtained with [^{3}H]spiperone were considered. N.D.; not determined.

3.3. Binding to 5-HT_{2A} receptors

In these experiments, fananserin bound to the 5-HT_{2A} receptor in rat cortical membranes labeled with [^{3}H]ketanserin with a K_i value of 0.37 nM, compatible with that published previously (Doble et al., 1992b). Of the other compounds tested, only risperidone and ritanserin showed comparable affinity (Table 1). Hill coefficients of these inhibition curves were generally close to unity.

3.4. Binding of [^{3}H]fananserin

[^{3}H]Fananserin bound to membranes from cells expressing recombinant human dopamine D_4 receptors in a reversible and saturable fashion (Fig. 2). The saturation curve could be fitted by a classical Langmuir isotherm, consistent with binding to a single class of binding sites over the concentration range used. This was supported by

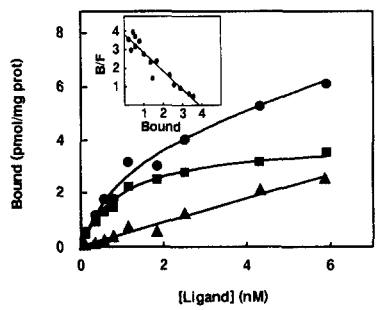

Fig. 2. Binding of [^{3}H]fananserin to human recombinant dopamine D_4 receptors. Saturation analysis of the binding of [^{3}H]fananserin to human recombinant dopamine D_4 receptors expressed in Chinese hamster ovary cells. The data are from one representative experiment performed in triplicate which was repeated twice more with similar results. Total binding is represented by ●, specific binding by ■ and non-specific binding by ▲. The line through the points represents the best fit to a simple Langmuir isotherm calculated by computer-assisted non-linear regression analysis. The inset shows a Scatchard transformation of the same data. Fifteen concentrations of [^{3}H]fananserin were used (0.05–15 nM).

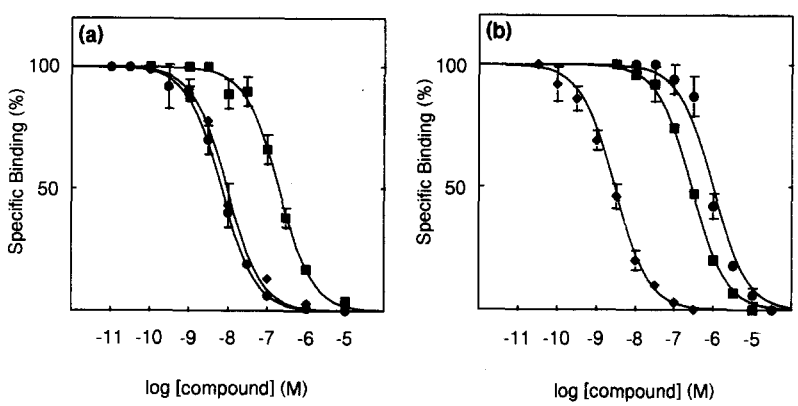

Fig. 1. Displacement of [^{3}H]spiperone binding to human recombinant dopamine D_4 receptors and dopamine D_2 receptors. Displacement curves for the inhibition by fananserin (●), clozapine (■) and haloperidol (♦) of the specific binding of [^{3}H]spiperone to recombinant human dopamine D_4 receptors expresssed in chinese hamster ovary cells (a) and to rat striatal dopamine D_2 receptors (b). The data represent the means ± S.E.M. of data from three independent determinations, each performed in duplicate (D_4) or triplicate (D_2). The line through the points represents the best fit to a simple Langmuir isotherm calculated by computer-assisted non-linear regression analysis.

Scatchard transformation of the data, which yielded linear plots. The apparent dissociation equilibrium constant (K_D) and the binding capacity (B_{max}) determined from the Scatchard plots were 0.725 ± 0.097 nM and 4.10 ± 0.09 pmol (mg protein)$^{-1}$, respectively ($n = 4$). The binding capacity using [^{3}H]fananserin as a ligand was similar to that obtained using [^{3}H]spiperone ($B_{max} = 3.4$ pmol (mg protein)$^{-1}$; data from Receptor Biology). The affinities of the different antipsychotics for the [^{3}H]fananserin binding sites on these cells are similar to those for the [^{3}H]spiperone sites (Table 1). The correlation coefficient, r, for the relationship between log K_i vs. [^{3}H]spiperone and log K_i vs. [^{3}H]fananserin for the six compound studied was 0.95. In rat brain, [^{3}H]fananserin principally labels the 5-HT$_{2A}$ receptor (Doble et al., 1992a; Malgouris et al., 1993).

4. Discussion

These data show that fananserin has high affinity for the human recombinant dopamine D_4 receptor, and indeed is one of the most potent compounds ever found to interact with this receptor subtype (Van Tol et al., 1991). Data demonstrating the antagonist activity of fananserin at dopamine D_4 receptors will be published elsewhere. Of particular interest is the observation that fananserin discriminates between dopamine D_4 and D_2 receptors. This selectivity is much greater (approximately 250 times) than that of clozapine [2 times in our study, 10 times in the original study by Van Tol et al. (1991)], and no other compound has been reported to have selective affinity for the dopamine D_4 receptor. Other such compounds do, however, exist, such as NGD 94-1 (Meade et al., 1995), YM 43611 ((*S*)-*N*-(1-benzyl-3-pyrrolidinyl)-5-chloro-4-cyclopropyl-carbonylamino-2-methoxybenzamide; Ohmori et al., 1995), L-745,870 (3-{[4-(4-chlorophenyl)piperazin-1-yl]methyl}-1*H*-pyrrolo[2,3-b]pyridine hydrochloride); Ragan, 1996) and U 93363E (3-ethoxy-*N*-methyl-*N*-[1-(phenylmethyl)-4-piperidinyl]-2-pyridinamine; Poel et al., 1995), but, to our knowledge, this information has only been published in abstract form.

Fananserin may, thus, be an interesting pharmacological tool with which to identify dopamine D_4 receptors in the central nervous system, and effects mediated thereby. Nonetheless, it should be pointed out that the interpretation of in vivo data may be compromised by the affinity of fananserin for 5-HT$_{2A}$ receptors. In our previous paper (Doble et al., 1992b), it was indicated that fananserin does not block bucco-facial stereotypies evoked by *d*-amphetamine, but this has been the only data published on the dopaminergic system. A more complete behavioural profile of fananserin in rats will be published elsewhere. In primates, fananserin reduces cognitive perseveration in response to psychostimulants, which may be indicative of potential antipsychotic activity in man (Mason et al., 1993).

The dopamine D_2 receptor subtype has long been considered to be the major site of action of classical antipsychotic drugs. It is probable that such activity is sufficient to treat at least the positive symptoms of schizophrenia, since substituted benzamide antipsychotics, such as raclopride, are extremely selective for this receptor. Clozapine may be an exception to this, since, at therapeutic concentrations, only low levels of dopamine D_2 receptor occupancy are reached (Farde et al., 1992; Seeman, 1992). Recently, binding studies with clozapine have revealed its high affinity for the dopamine D_4 receptor, and it was suggested that this receptor subtype might be the therapeutic target of clozapine (Van Tol et al., 1991). It has not been possible to evaluate this hypothesis up till now, since no other dopamine D_4 receptor selective drugs existed which were largely devoid of activity at dopamine D_2 receptors. The dopamine receptor selectivity of fananserin make it a suitable agent with which to assess the potential antipsychotic effect of dopamine D_4 receptor blockade. Furthermore, the low affinity of fananserin for the dopamine D_2 receptor may result in reduced extrapyramidal and neuroendocrine side-effects.

In terms of its potential antipsychotic activity, another interesting characteristic of fananserin lies in its potent antagonist activity on 5-HT$_{2A}$ receptors. Activity at this latter receptor may be useful in improving negative symptoms of schizophrenia, as has been suggested, for example for risperidone and sertindole (Borison, 1995; Meltzer et al., 1989; Roth et al., 1995).

Fananserin, thus, offers an original in vitro profile of monoamine receptor binding, high affinity for D_4 and 5-HT$_{2A}$ receptors and low affinity for dopamine D_2 receptors. It provides an opportunity to assess the role of dopamine D_4 receptors in the central nervous system, and their potential as a therapeutic target in innovative treatments for schizophrenia.

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