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European Journal of Pharmacology 322 (1997) 283–286

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Substituted 4-aminopiperidines having high in vitro affinity and selectivity for the cloned human dopamine D_4 receptor

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Received 19 December 1996; accepted 24 December 1996

Abstract

We have discovered two substituted 4-aminopiperidine compounds having high in vitro affinity and selectivity for the human dopamine D_4 receptor. Both compounds, 3-ethoxy-*N*-methyl-*N*-[1-(phenylmethyl)-4-piperidinyl]-2-pyridinylamine (U-99363E), and its 3-isopropoxy analog (U-101958), were found through a routine receptor binding screen. The determined affinities (K_i) of these compounds for the cloned human dopamine D_4 receptor were 2.2 and 1.4 nM, respectively. They exhibited at least 100-fold lower affinities for dopamine D_2 and for other dopaminergic, serotonergic and adrenergic receptors. Both compounds were found to antagonize quinpirole-induced mitogenesis in Chinese hamster ovary cells expressing the human dopamine D_4 receptor. In spite of their poor metabolic stability and low bioavailability, U-99363E and U-101958 appear to be among the first high-affinity, highly selective dopamine D_4 receptor antagonists reported, and may have utility in in vitro investigations requiring selective tagging or blockade of dopamine D_4 sites.

Keywords: Dopamine D_4 receptor, human; Receptor binding; Aminopiperidine

1. Introduction

Pharmacological and physiological studies, as well as abundant clinical experience strongly support a role for dopamine receptors in neurotransmission within the central nervous system. The contributions of the individual dopamine receptor subtypes to these various functions are the subject of intense research. In support of this work, the cloning of dopamine receptor subtypes is providing opportunities for the discovery of subtype-selective agonists and antagonists. These selective drugs will in turn be useful for the elucidation of the relative functions of dopamine receptor subtypes within the central nervous system, and for more effective treatments for mental, hormonal and motor disturbances stemming from aberrant dopamine neurotransmission.

The dopamine D_4 receptor subtype has been proposed to play a role in adenylate cyclase-mediated signaling events (Cohen et al., 1992) and in the regulation of melatonin synthesis in the vertebrate retina (Zawilska, 1994). Based on the localization and abundance of dopamine D_4 receptor mRNA in the heart, a possible role for this receptor subtype in cardiovascular function has been argued (O'Malley et al., 1992). Further, it has been suggested that a blockade of central dopamine D_4 receptors may participate directly in the efficacy of antischizophrenic drugs (Van Tol et al., 1991; Seeman and Van Tol, 1993). Further development of these ideas is hampered by a preponderance of dopamine D_2 receptor sites in brain regions occupied by the dopamine D_4 receptor (Murray et al., 1995) and the lack of pharmacological agents truly selective for this receptor subtype.

We report the discovery of two closely related substituted 4-aminopiperidine compounds having high affinity and selectivity for the human dopamine D_4 receptor. The antagonist properties of these compounds were determined by their ability to block dopamine D_4 receptor-mediated signal transmission in vitro.

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0014-2999/97/$17.00
PII S0014-2999(97)00013-7

2. Materials and methods

2.1. Materials

Radioligands were obtained either from Amersham or New England Nuclear. Drugs were purchased from Research Biochemicals International or Sigma.

2.2. Receptor binding methods

The binding profiles of 3-ethoxy-*N*-methyl-*N*-[1-(phenylmethyl)-4-piperidinyl]-2-pyridinylamine (U-99363E), and its 3-isopropoxy analog (U-101958) were initially evaluated in duplicate at a single concentration, 1 μM, in a battery of ten assays as part of a routine screening effort. Where the results of these initial assays suggested activity (greater than 25% inhibition), full dose-response experiments were performed using 11 half-log dilutions of drugs run in duplicate tubes. The radioligands (all tritiated) used both in the initial assays and in the dose-response studies, were prazosin (adrenergic α_1 sites, 76 Ci/mmol, 1.2 nM), clonidine (adrenergic α_2 sites, 60 Ci/mmol, 3.8 nM), SCH 23390 (dopamine D_1 sites, 71 Ci/mmol, 0.3 nM), U-86170 (dopamine D_2 sites, 62 Ci/mmol, 1–2 nM, Moon and Hsi, 1992), spiperone (dopamine D_3 and D_4 sites, 97 Ci/mmol, 0.7 nM), 8-hydroxy-2-(di-*n*-propylamino)tetralin (5-HT_{1A} sites, 85 Ci/mmol, 1.2 nM) and ketanserin (5-HT_2 sites, 62 Ci/mmol, 0.8 nM). Non-specific binding (5–20% of total) was determined with 3 μM of the following cold compounds (listed in the same order as the radioligands above): phentolamine, clonidine, SCH 23390, haloperidol (D_2, D_3 and D_4), lisuride and spiperone. The sources of binding sites were as follows: homogenized rat cortex (α_1, α_2 sites); homogenized rat striatum (D_1 sites); Chinese hamster ovary (CHO) cell membranes prepared from cells expressing the rat dopamine D_{2i} receptor (Chio et al., 1990), the rat dopamine D_3 receptor (Chio et al., 1994a) or the human 5-HT_{1A} receptor (Fargin et al., 1988); and human embryonic kidney cell membranes from cells expressing the human dopamine $D_{4.2}$ receptor (Chio et al., 1994b). Buffers used were 50 mM Tris, 5 mM $MgCl_2$, pH 7.4 (for α_1, α_2, 5-HT_{1A} and 5-HT_2 receptor binding), 50 mM Tris, 120 mM NaCl, 5 mM KCl, 5 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4 (for D_1 receptor binding); 20 mM HEPES, 10 mM $MgSO_4$, pH 7.4 (for D_2 receptor binding); and 20 mM HEPES, 10 mM $MgSO_4$, 150 mM NaCl, 1 mM EDTA, pH 7.4 (for D_3 and D_4 receptor binding). Incubation of the 0.9 ml binding reactions was for 1 h at room temperature. Reactions were stopped by vacuum filtration using ice-cold 50 mM Tris, 5 mM $MgCl_2$, pH 7.4. IC_{50} values were estimated by fitting the data to a one-site model by non-linear least squares minimization using GraphPad Prism. K_i values were calculated according to the Cheng–Prusoff equation (Cheng and Prusoff, 1973). These are expressed in Table 1 with 95% confidence intervals for the weighted mean value constructed using individual standard deviations (Finney, 1978). In cases where the drug failed to produce 25% inhibition at 1 μM concentration in the initial study, dose-response studies were not performed and the IC_{50} was estimated to be greater than 1 μM.

2.3. Mitogenesis methods

CHO cells expressing the human dopamine D_4 receptor were seeded into 96-well plates and grown at 37C for 48 h in alpha minimum essential medium with Earle's salts (Irvine Scientific) supplemented with 10% fetal calf serum. The wells were rinsed twice with serum-free medium and fresh medium (or medium containing the dopamine agonist quinpirole (30 nM)) was added, along with 10 μl of the test compound or sterile water alone. After culture for

Table 1
Receptor binding data for U-99363(E), U-101958, clozapine and raclopride

Receptor	Binding constant, K_i (nM with 95% confidence intervals)			
	U-99363E	U-101958	Clozapine [c]	Raclopride [c]
Dopamine D_4	2.2 (1.9–2.5)	1.4 (1.2–1.6)	32 (30–35)	1549 (1207–1987)
	$n = 3$	$n = 2$	$n = 5$	$n = 3$
Dopamine D_1	> 1000 [a]	> 1000 [a]	n.d. [b]	n.d.
Dopamine D_2	278 (226–342)	648 (500–840)	55 (46–65)	1.0 (0.9–1.1)
	$n = 4$	$n = 2$	$n = 2$	$n = 2$
Dopamine D_3	1291 (926–1799)	2528 (1761–3628)	n.d.	n.d.
	$n = 2$	$n = 2$		
5-HT_{1A}	> 3750	> 3750	n.d.	n.d.
5-HT_{2A}	508 (406–636)	300 (232–388)	n.d.	n.d.
	$n = 3$	$n = 2$		
Adrenergic α_1	> 1000 [a]	> 1000 [a]	n.d.	n.d.
Adrenergic α_2	> 1000 [a]	> 1000 [a]	n.d.	n.d.

[a] IC_{50} values estimated based on testing drugs at 1000 nM concentration. In these cases, this test resulted in less than 25% competition.
[b] n.d., not done.
[c] Data from Lawson et al. (1994).

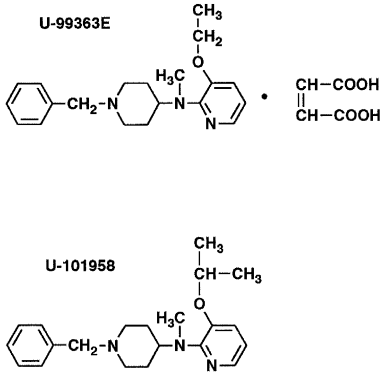

Fig. 1. Chemical structures of U-99363E and U-101958. This report contains data both for U-99363, the free base, and U-99363E, the butenedioate salt drawn in the figure.

16–18 h, [^{3}H]thymidine (1 μCi/well) was added and the plates were further incubated for 2 h. The cells were trypsinized and harvested onto filter mats using a TomTec plate harvester. Filters were counted in a BetaPlate scintillation counter (Pharmacia).

3. Results

3.1. Receptor binding studies

During routine screening for compounds having in vitro binding affinity for dopamine or serotonin receptors, two substituted 4-aminopiperidine compounds (Fig. 1) were found to compete very effectively for binding at the human dopamine D_4 receptor, but relatively poorly for other dopaminergic, serotonergic and adrenergic receptors. Full dose-response studies placed the dopamine D_4 affinities (K_i) of U-99363E and U-101958 at 2.2 and 1.4 nM, respectively, while their affinities for the other receptors were at least 100-fold lower (Table 1). Most notably, U-99363E and U-101958 had 126- and 462-fold higher affinity for the dopamine D_4 than for the D_2 receptor.

For comparison, Table 1 also contains dopamine D_4 and D_2 binding data for the dopamine antagonists clozapine and raclopride. Clozapine, as reported previously (Lahti et al., 1993; Mills et al., 1993), had very little dopamine D_4 versus D_2 receptor selectivity. Raclopride, by contrast, had greater than 1000-fold selectivity for the dopamine D_2 subtype (Van Tol et al., 1991; Lahti et al., 1993).

3.2. Mitogenesis studies

Like the dopamine antagonist clozapine, U-99363E and U-101958 (1 μM) were found to inhibit quinpirole-induced mitogenesis in the CHO cell line expressing the human dopamine D_4 receptor (Fig. 2). Raclopride failed to inhibit quinpirole-induced mitogenesis in this cell line, consistent with its very low affinity for dopamine D_4 sites.

4. Discussion

The binding affinities of U-99363E and U-101958 place them among the first high-affinity, selective dopamine D_4 receptor antagonists reported. Clozapine, originally reported to have a high degree of dopamine D_4 versus D_2 receptor selectivity (Van Tol et al., 1991), has been shown in subsequent studies to have at best very modest receptor selectivity (Lahti et al., 1993; Lawson et al., 1994). Even the more highly selective pyridobenzodiazepine derivative of clozapine, JL 18, has only approximately 25-fold dopamine D_4 versus D_2 receptor selectivity (Liégeois et al., 1995). By contrast, the potencies of U-99363E and U-101958 and their selectivities are more comparable to

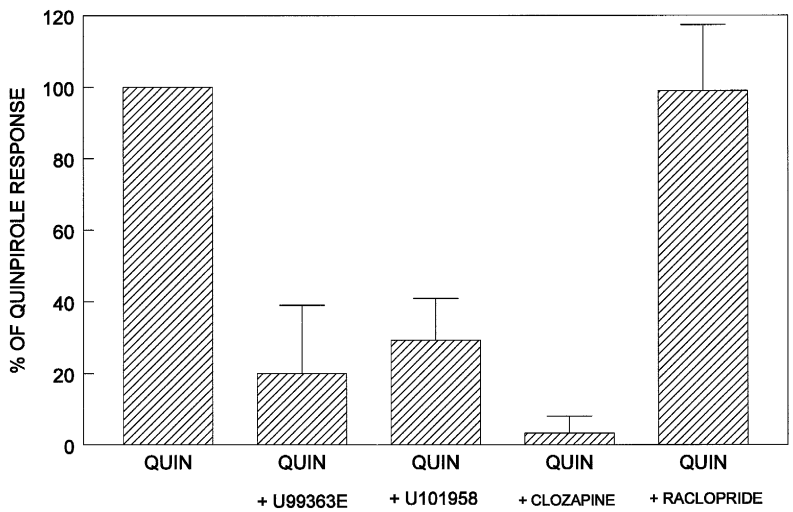

Fig. 2. Antagonism of quinpirole-induced mitogenesis in CHO cells expressing the cloned human dopamine D_4 receptor. Quinpirole (30 nM) was added to cells in triplicate plates, along with test compound (1 μM) or sterile water 16–18 h prior to the addition of [^{3}H]thymidine (1 μCi/well). After 2 h further incubation, the cells were released from the plates, harvested onto filtermats, and counted. The error bars represent standard deviations. For U-99363E and U-101958, $n = 3$; for clozapine and raclopride, $n = 1$.

NGD 94-1 (Neurogen), reported to have a dopamine D_4 affinity of 3.6 nM and approximate 750-fold selectivity over D_2 receptor affinity (Meade et al., 1995).

Unfortunately, both U-99363E and U-101958 have been found to have clinically insufficient metabolic stability when incubated with rat liver hepatocytes, and the i.v. versus oral bioavailability of U-99363E in monkeys is approximately 1% (data not shown). Thus, the expected utility of these compounds for the assessment of dopamine D_4 receptor localization and function in vivo is very poor.

In in vitro studies requiring agents providing a selective tag or blockade of dopamine D_4 sites, however, U-99363E and U-101958 may be very useful.

Acknowledgements

The authors thank M.E. Torkelson and J.K. Zelenock for technical and administrative assistance.

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