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Modulation of the clozapine structure increases its selectivity for the dopamine D_4 receptor

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Abstract

Clozapine has a more marked affinity for the recently cloned dopamine D_4 receptor than for the dopamine D_2 receptor. In the search for a selective ligand for the dopamine D_4 receptor, useful as a pharmacological tool or as a potent atypical antipsychotic, a pyridobenzodiazepine derivative bioisoster of clozapine, JL 18, 8-methyl-6-(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine, was found to be the most dopamine D_4-selective ligand belonging to the diarylazepine class. Indeed, JL 18 binds to the dopamine D_4 receptor with affinity up to 25 times superior to that for the dopamine D_2 receptor and presents reduced affinities for other receptors.

Keywords: Dopamine D_4 receptor; Clozapine; Pyridobenzodiazepine

Although neuroleptic drugs are generally antidopaminergic compounds with a high affinity for the dopamine D_2 receptor subtype, a selective blockade of limbic dopaminergic neurons is thought to be responsible for the therapeutic action while it appears that the dopamine D_2 blockade in striatum and in the tuberoinfundibular system, respectively generates extrapyramidal side-effects such as akathisia or tardive dykinesia and neuroendocrine disorders.

However, some molecules present good antipsychotic efficacy with a low propensity for inducing extrapyramidal side-effects. The best example is clozapine, the leader among atypical antipsychotic compounds (Bruhwyler et al., 1990). Clozapine is a weak antidopaminergic D_2 agent but is more selective for the dopamine D_4 receptor (Van Tol et al., 1991) (Table 1). Indeed, molecular biology procedures have demonstrated the multiplicity of dopamine receptors by discovering at least five cloned dopamine receptors (Seeman and Van Tol, 1994). Dopamine D_4 receptors could therefore constitute a new target for original antipsychotic drugs. The importance of dopamine D_4 receptors in schizophrenic disorders is reinforced by the fact that dopamine D_4 receptor density is elevated by 600% in schizophrenic patients while the number of dopamine D_2 (or D_3) receptors was only increased by 15% (Seeman et al., 1993).

Nevertheless, clozapine also possesses high affinity for serotonin 5-HT_2, α_1-adrenoceptor, muscarinic acetylcholine and histamine H_1 receptors that could generate its well-known side-effects (orthostatic hypotension, tremor, seizures, hypersalivation, ...). Thus, it appears important both from a pharmacological and a therapeutic point of view to identify newer antagonists which are more dopamine D_4-selective than clozapine.

In the search for new atypical antipsychotics, a series of pyridine isosters of clozapine has been developed (Liégeois et al., 1993) and biologically evaluated using in vitro and in vivo procedures (Bruhwyler et al., 1992; Liégeois et al., 1993). Among these original compounds, some molecules revealed very promising antipsychotic activities and, therefore, were selected for further investigations. One of them, JL 18, 8-methyl-6-

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Table 1
Binding profile and selectivity of JL 18, clozapine and (+)-*N*-propyl-norapomorphine

Compound	K_i (nM)			K_i (nM)		
	D_2	D_4 [b]	D_2/D_4	5-HT_2 [a]	D_1 [a]	M [a]
JL 18	530 [a]	21	*25.24*	94	398	48
Clozapine	45 [a]	21 [d]	*2.14*	3.8	115	25
Clozapine	230 [d]	21 [d]	*10.95*	–	–	–
Clozapine	82 [c]	29 [c]	*2.83*	–	–	–
(+)-*N*-Propyl-norapomorphine	257 [e]	13 [e]	*19.8*	–	–	–
(+)-*N*-Propyl-norapomorphine	291 [c]	120[c]	*2.42*	–	–	–

Experiments done 2–3 times; S.E. of K_i values, ±10%. [a] From Bruhwyler et al. (1992); Liégeois et al. (1993). [b] Determined according to the methodology of Van Tol et al. (1991). [c] From Lahti et al. (1993). [d] From Seeman and Van Tol (1994). [e] From Seeman and Van Tol (1993).

(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine, was tested according to the previously described methodology of Van Tol et al. (1991) in order to determine its dopamine D_4 affinity. The human dopamine D_4 receptors are transiently expressed in COS-7 cells (Van Tol et al., 1991).

Briefly, the competition between JL 18 and [^{3}H]spiperone for binding at D_4 receptors was evaluated using tissue culture cells, scraped from Petri dishes and homogenized by hand in a Teflon-glass homogeniser. The homogenates were centrifuged for 15 min at 39 000 × *g*, and the pellets were resuspended in buffer to a final concentration of 150–250 μg protein/ml. Each tube received 0.5 ml buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 120 mM NaCl), 0.5 ml [^{3}H]spiperone (final concentration of 250 pM; 60–100 Ci/mmol; Amersham), and 0.5 ml cell membrane suspension. After incubation for 2 h at room temperature, the incubates were filtered. Non-specific binding was defined in the presence of 30 μM dopamine. The dissociation constant, K_i, was derived from the concentration, *C*, for 50% inhibition of binding, using $K_i = C/(1 + C^*/K_d)$, where C^* was 250 pM [^{3}H]spiperone, and where the K_d for [^{3}H]spiperone was 88 pM for the dopamine $D_{4.2}$ receptor (variant of the human dopamine D_4 receptors). Dopamine D_2, serotonin 5-HT_2, dopamine D_1 and muscarinic acetylcholine receptor affinities were determined according to the well-known procedures (for details see Bruhwyler et al., 1992; Liégeois et al., 1993). Each K_i value was obtained at least in duplicate with 6–9 concentrations of the drug in triplicate. The D_4/D_2 binding ratio was also calculated using K_i values. The results are reported in Table 1 in which binding results obtained from different laboratories are presented for comparison. Indeed, in vitro procedures are very sensitive to several parameters, and therefore, great variations are frequently seen in the literature.

JL 18 showed a dopamine D_4 affinity close to that of clozapine. Moreover, JL 18 was a weak dopamine D_2 agent and presented lower muscarinic acetylcholine and serotonergic affinities than clozapine (Bruhwyler et al., 1992; Liégeois et al., 1993). The 25-fold greater selectivity of JL 18 for the dopamine D_4 receptor than for the dopamine D_2 receptor was higher than the 2-fold selectivity of clozapine (Table 1). Lahti et al. (1993) also found a 2-fold selectivity of clozapine while a 10-fold selectivity of clozapine was also reported (Van Tol et al., 1991) (Table 1). However, as mentioned above, JL 18 presents lower affinities for other binding sites – serotonin 5-HT_2, dopamine D_1, muscarinic acetylcholine – compared to clozapine (Liégeois et al., 1993), and, therefore, further demonstrates its selectivity for dopamine D_4 receptors.

The selectivities of different agonist and antagonist enantiomers for dopamine D_2 and D_4 receptors have already been described (Seeman and Van Tol, 1993). (+)-*N*-Propyl-norapomorphine appeared as the most selective ligand with affinities (K_i) of 13 and 257 nM for dopamine D_4 and D_2 receptors, respectively. However, this 20-fold selectivity for the dopamine D_4 receptor over the dopamine D_2 receptor has recently been contradicted (Lahti et al., 1993). (+)-*N*-Propyl-norapomorphine is a potent and regionally highly selective limbic dopaminergic antagonist but with a low bioavailability. This parameter was improved by synthesizing other analogues such as 10,11-dioxymethylene-*N*-propyl-norapomorphine or 11-hydroxy-*N*-propyl-norapomorphine but as reported (Lahti et al., 1993; Seeman and Van Tol, 1993) they did not possess a better D_4 selectivity. In contrast with (+)-*N*-propyl-norapomorphine, JL 18 has been shown to be active in a range of doses close to that for clozapine in two behavioural models: open-field test in the rat using the intraperitoneal route and temporal regulation conditioning schedule in the dog using the oral route (Bruhwyler et al., 1992).

On the basis of these preliminary results, further neurochemical and behavioural investigations are necessary to firmly establish the pharmacological interest of this original compound and also the exact implication of D_4 receptors in the etiology of psychotic diseases.

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