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Short communication

Novel insights on the structural determinants of clozapine and olanzapine multi-target binding profiles

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ABSTRACT

The clinical efficacy of antipsychotic drugs has been associated with a certain binding profile for a set of G protein-coupled receptors (GPCR)s. In this work, we use the structurally-related clozapine–olanzapine pair to progress in the understanding of the structural properties that determine their divergent binding profiles and, thereby, their differing therapeutic efficacy. First, we present novel site-directed mutagenesis results that confirm our previous hypothesis on the importance of ligand interaction with positions 5.42 and 5.46 in transmembrane helix 5. Then, we use refined models of ligand-receptor complexes, built from recently published GPCR crystal structures, to gain further insight into the molecular mechanisms responsible for the observed experimental outcomes. In particular, we observe that preventing or potentiating hydrogen bonding with position 5.46, could allow obtaining ligands with, respectively, clozapine or olanzapine-like affinities. Results presented in this study could guide the design of antipsychotic candidates with tailored binding profiles.

1. Introduction

Currently used antipsychotic drugs are characterized by having complex pharmacological profiles. In particular, it is widely accepted that drugs from this class can interact with a variety of G protein-coupled receptors (GPCRs), and that this set of interactions is responsible both for their therapeutic as well as for some of their associated side-effects. Attempts to disentangle wanted from unwanted effects by designing more selective candidates have failed to produce compounds retaining the therapeutic efficacy observed in promiscuous drugs. This has led some authors to postulate that drugs possessing the right combination of binding affinities for several drug targets could be the solution for the development of new treatments against schizophrenia [1]. There is still no consensus about which receptors constitute antipsychotic therapeutic targets or anti-targets [2], nonetheless, the study of the binding profiles of drugs with a proven efficacy could provide a good starting point for further investigation.

An additional difficulty for the rational design of novel antipsychotic candidates is the current lack of knowledge about the structural features determining drug binding profiles at GPCRs. Unveiling these structural determinants is a critical step for the development of new drugs with a tailored binding affinity profile [3,4]. In this sense, the growing amount of structural information on GPCRs is opening a new avenue for the study of the structural determinants governing the binding preferences of promiscuous compounds.

From the available antipsychotic drugs to date, clozapine has proven to be the most efficacious treatment option in several clinical trials [5]. Unfortunately, clozapine presents life-threatening side-effects related to the production of toxic metabolized species [6] and, for that reason, it is not the first treatment of choice for schizophrenic patients. Olanzapine, on the other hand, despite being less efficacious, has been proposed to be the second best option in terms of efficacy [7]. Even if the structures of clozapine and olanzapine are very similar, their binding affinity profiles for diverse GPCRs show remarkable differences, which could be responsible for the lower therapeutic efficacy of olanzapine

Abbreviations: GPCRs, G protein-coupled receptors; TM5, transmembrane helix 5; WT, *wild type*.

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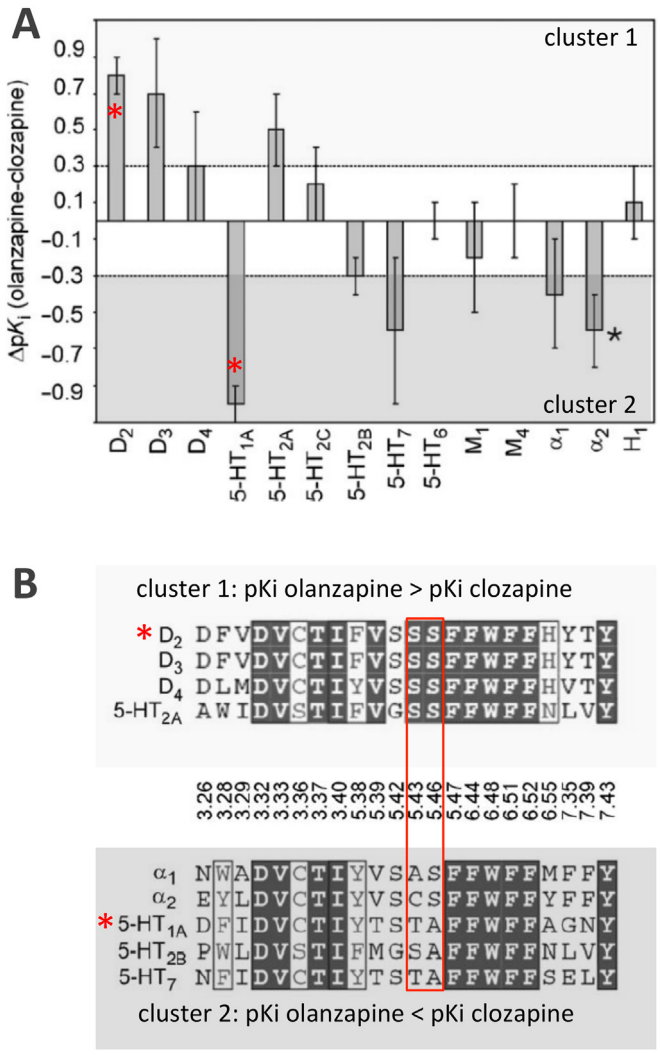

Fig. 1. (A) Differences in binding affinities of clozapine and olanzapine plotted as pKi (olanzapine-clozapine) for a set of antipsychotic-relevant GPCRs. Receptors showing differences of >0.3 log units in pKi where assigned to one of the following clusters: cluster 1 contains receptors with pKi olanzapine > pKi clozapine (D_2, D_3, D_4, 5-HT_{2A}); cluster 2 contains receptors with pKi olanzapine < pKi clozapine (α_1, α_2, 5-HT_{1A}, 5-HT_{2B}, 5-HT_7). (B) Multiple sequence alignment of clozapine and olanzapine receptor targets is clustered according to drug preference in (A). GPCRs with highest preference for clozapine and olanzapine are marked with a red asterisk. A black asterisk indicates that binding was determined using rat α_2 receptor. Figure is adapted from reference [8]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 1A). Therefore, the clozapine/olanzapine pair is an excellent system to investigate how subtle structural differences between drugs can affect binding profiles, and how these changes are translated into clinically-relevant effects.

In a previous attempt to identify the structural determinants of clozapine and olanzapine binding profiles, our group built structural 3D complexes of these drugs with several GPCRs [8]. The resulting docking poses allowed selecting residues in the receptor binding sites which were used to perform a multiple sequence alignment (see Fig. 1B, residue numbering according to reference [9]). The obtained alignment evidences the presence of a conserved double-serine at positions 5.43 and 5.46 in the cluster of receptors showing a preference to bind olanzapine over clozapine (cluster 1, Fig. 1B), which is not present in the cluster of receptors with a preference for clozapine (cluster 2, Fig. 1B). These sequence conservation differences led us to suggest that interaction with residues in these positions could be a key modulator of the differential affinities of clozapine and olanzapine.

Table 1
Affinity constants of clozapine and olanzapine at the dopamine D_{2S} and D_{2S}-S5.43T/S5.46A receptors.

	D_{2S}		D_{2S}-S5.43T/S5.46A	
	Ki (nM)	pKi	Ki (nM)	pKi
Clozapine	88.65 ± 10.04, $N = 5$	7.05	58.04 ± 9.65, $N = 3$	7.24
Olanzapine	23.45 ± 4.38, $N = 5$	7.63	173.90 ± 31.5, $N = 4$	6.76

With the aim to experimentally validate this hypothesis, in this study, we performed site-directed mutagenesis. The experimental outcome was rationalized using novel computational models taking advantage of recently published crystal structures. Our study provides novel insights into structural determinants that drive the divergent binding profile of clozapine and olanzapine and have important implications for the design of improved antipsychotic agents.

2. Results and discussion

Site-directed mutagenesis was performed to determine the importance of positions 5.43 and 5.46 for drug selectivity. Our intention was to alter the binding properties of cluster 1 receptors (with more affinity for olanzapine) and make them more similar to cluster 2 receptors (with more affinity for clozapine). To do so, we mutated the residues found in the most olanzapine-selective receptor (the D_2 receptor, with S5.43 and S5.46) into the residues found in the most clozapine-selective receptor (the 5-HT_{1A} receptor, with T5.43 and A5.46). If our hypothesis was correct, the

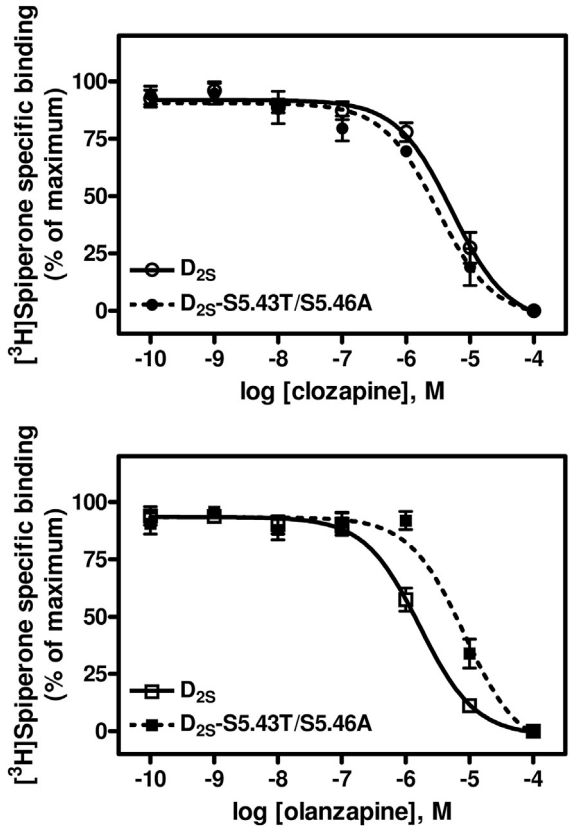

Fig. 2. Competitive binding curves for clozapine and olanzapine at the *wild type* (solid line) and mutated D_2 receptor (dashed line).