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Molecular targets of atypical antipsychotics: From mechanism of action to clinical differences

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ABSTRACT

The introduction of atypical antipsychotics (AAPs) since the discovery of its prototypical drug clozapine has been a revolutionary pharmacological step for treating psychotic patients as these allow a significant recovery not only in terms of hospitalization and reduction symptoms severity, but also in terms of safety, socialization and better rehabilitation in the society. Regarding the mechanism of action, AAPs are weak D_2 receptor antagonists and they act beyond D_2 antagonism, involving other receptor targets which regulate dopamine and other neurotransmitters. Consequently, AAPs present a significant reduction of deleterious side effects like parkinsonism, hyperprolactinemia, apathy and anhedonia, which are all linked to the strong blockade of D_2 receptors.

This review revisits previous and current findings within the class of AAPs and highlights the differences in terms of receptor properties and clinical activities among them. Furthermore, we propose a continuum spectrum of "atypia" that begins with risperidone (the least atypical) to clozapine (the most atypical), while all the other AAPs fall within the extremes of this spectrum.

Clozapine is still considered the gold standard in refractory schizophrenia and in psychoses present in Parkinson's disease, though it has been associated with adverse effects like agranulocytosis (0.7%) and weight gain, pushing the scientific community to find new drugs as effective as clozapine, but devoid of its side effects. To achieve this, it is therefore imperative to characterize and compare in depth the very complex molecular profile of AAPs. We also introduce relatively new concepts like biased agonism, receptor dimerization and neurogenesis to identify better the old and new hallmarks of "atypia".

Finally, a detailed confrontation of clinical differences among the AAPs is presented, especially in relation to their molecular targets, and new means like therapeutic drug monitoring are also proposed to improve the effectiveness of AAPs in clinical practice.

Contents

Abbreviations: 5-HT, Serotonin; AAP, Atypical antipsychotic; ACh, Acetylcholine; Akt, Protein kinase B; AP, Antipsychotic; BDNF, Brain-derived neurotrophic factor; CIAS, Cognitive impairment associated with schizophrenia; CNS, Central nervous system; Cp, Plasma concentration; EPS, Extrapyramidal symptoms; ERK1/2, Extracellular signal-regulated kinases 1 and 2; FGF2, Fibroblast growth factor; GlyT, Glycine transporter; GPCR, G protein-coupled receptor; GSK3, Glycogen synthase kinase 3; k_{off}, Receptor dissociation constant; k_{on}, Receptor association constant; mGlu, Metabotrobic glutamate; mPFC, Medial prefrontal cortex; NAc, Nucleus accumbens; NGF, Nerve growth factor; NMDA, N-methyl-D-aspartate; PCP, Phencyclidine; PD, Parkinson's disease; PET, Positron emission tomography; PFC, Prefrontal cortex; P-gp, P-glycoprotein; TAP, Typical antipsychotic; TD, Tardive dyskinesia; TDM, Therapeutic drug monitoring; VTA, Ventral tegmental area.

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1. Introduction

Antipsychotics (APs) are commonly prescribed drugs for treating schizophrenia, bipolar disorder and other brain diseases that are characterized by psychotics features (Miyamoto, Miyake, Jarskog, Fleischhacker, & Lieberman, 2012). Generally, these drugs are divided into typical antipsychotics (TAPs), referred to as first generation drugs, and atypical antipsychotics (AAPs), referred to as second-generation drugs, based on the concept that AAPs have reduced side effects such as parkinsonism and tardive dyskinesia (TD) (Meltzer, 2013), and eventually a better profile in terms of social and cognitive improvement. However, this distinction has been questioned by different authors (Gründer, Hippius, & Carlsson, 2009; Keefe et al., 2004), suggesting that each AP is unique.

In the 1970's, the AAPs were introduced through clozapine, a prototypical drug that was demonstrated to be very effective not only for treating the positive symptoms of schizophrenia, but also to reduce the negative and cognitive problems associated with the disorder, including a strong reduction of motor-related side effects (Wenthur & Lindsley, 2013). Particularly, clozapine proved effective in patients resistant to other APs (Gillespie, Samanaite, Mill, Egerton, & MacCabe, 2017). As the introduction of TAPs has been revolutionary in reducing hospitalization and deaths of schizophrenic and psychotic patients, the discovery of clozapine, and subsequently of other AAPs, has resulted in significant recovery of these patients in terms of cognition and integrating into society. The benefits of clozapine have unfortunately been outweighed by its potential side effects, such as the risk of severe hematological effects (0.7%) and weight gain, and this has complicated its use compelling clinicians to perform mandatory drug and blood monitoring (Capannolo et al., 2015). For this reason, there has been a strong effort in the biomedical and pharmaceutical scientific community to find new drugs as effective as clozapine, but devoid of its relevant side effects.

Importantly, the success of clozapine and other AAPs introduced a new concept in relation to the mechanism of action, i.e. that drugs with low affinity for the dopamine D_2 receptor could be an effective AP through the involvement of other receptors, such as 5-HT_{2A} serotonin receptors. The involvement of serotonin (5-HT) receptors was an important step forward to understand the mechanism of actions of AAPs, and moreover the affinity ratio 5-HT_{2A}/D_2 was considered a hallmark for AAPs. Since then, considering that the 5-HT_{2A}/D_2 ratio hypothesis was not completely satisfactory as it was unable to thoroughly explain the differences among the AAPs, many hypotheses have been formulated on this topic (Miyamoto, Duncan, Marx, & Lieberman, 2005) trying to explain unambiguously the mechanism of "atypia". Indeed, for AAPs (including clozapine), many works have pointed out the importance of other G protein-coupled receptors (GPCRs), beyond D_2 and 5-HT_{2A}, such as serotonin (5-HT_{2C} and 5-HT_{1A}), muscarinic, noradrenergic, glutamatergic and histamine receptors (Meltzer & Massey, 2011). Besides GPCRs, other targets have also been considered, such as ion channels (e.g. N-methyl-D-aspartate (NMDA)), transporters (e.g. glycine transporters) and enzymes (e.g. glycogen synthase kinase 3 (GSK3)), in order to explain the characteristics of AAPs.

In addition, other parameters such as receptor dissociation (k_{off}) and association (k_{on}) kinetics have been taken into consideration (e.g. for D_2 receptor) to better understand the mechanism of AAPs, particularly for their side effects like parkinsonism and hyperprolactinemia.

Furthermore, although acute events, such as psychoses, are probably controlled by short-term effects of AAPs that are mostly mediated by their receptor affinities, it is evident that these drugs have more complex effects, particularly in the long term, involving intracellular mechanisms that may regulate neuronal functionality, neuroplasticity and neurogenesis (Fumagalli, Frasca, Racagni, & Riva, 2009; Molteni, Calabrese, Racagni, Fumagalli, & Riva, 2009) through the activation of proteins such as extracellular signal-regulated kinases 1 and 2 (ERK1/2) and protein Kinase B (Akt) (Freyberg, Ferrando, & Javitch, 2010).

In addition, new concepts related to GPCR function, such as "biased agonism" and receptor dimerization have been recently introduced, which have added further complexity and intrigue over the mechanism of action of AAPs. In fact, new studies have demonstrated how the activation of specific functions of the 5-HT_{2A} receptor can be responsible to distinguish clozapine and other AAPs from TAPs (Aringhieri et al., 2017; Mocci, Jiménez-Sánchez, Adell, Cortés, & Artigas, 2014). Other evidence has pointed out the relevance of the $5HT_{2A}$-mGlu2 receptor complex to determine the 5-HT_{2A} signaling properties and how clozapine might influence the heteromer activity (González-Maeso et al., 2008).

Besides pharmacodynamics, pharmacokinetics is another key determinant factor that can help to explain the therapeutic success and clinical differences among AAPs. Considering the variability in AAP metabolism among patients, therapeutic drug monitoring (TDM) can be particularly useful for *non-responders*, and also to reduce relevant side effects.

In conclusion, even after 40 years since the discovery of clozapine, we are still trying to understand: 1) Which are the hallmarks defining an ideal AAP and 2) Why clozapine, at least in terms of efficacy, is superior compared to other AAPs. Finally, this review proposes a plausible correlation between AAPs mechanism of action and their clinical differences. These aspects need proper investigation in order to find new ways to produce better drugs.

2. Classic mechanisms of action of atypical antipsychotics (AAPs)

2.1. Dopaminergic system and dopamine receptors

The dopaminergic system plays a key role in the pathogenesis of schizophrenia and related disorders. The "dopaminergic hypothesis" of schizophrenia postulates a hyperactivity of dopamine in the mesolimbic system and a hypofunctionality of dopamine in other brain areas like the prefrontal cortex (PFC) (Carlsson & Lindqvist, 1963; van Rossum, 1966).

Direct evidence of dopaminergic system dysfunction was found either with the use of radioligand compounds or by positron emission tomography (PET) ligands. In addition, a change in dopamine synthesis was determined in humans and animals by studying the uptake and storage of the dopamine precursor 18F-L-dopa. Taking advantage of these techniques, it was possible to show a hypersensitivity to amphetamine in schizophrenic patients, because an increase of dopamine release resulted in exacerbation of psychosis (Breier et al., 1997). Moreover, supporting this notion, the use of amphetamine is a well-established model of schizophrenia in animals, acting mostly by increasing dopamine release (Lillrank, Oja, Saransaari, & Seppälä,

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1991). Convincing data underline the relevance of increased dopamine synthesis and release in the pathogenesis of schizophrenia, as a consequence of altered presynaptic dopaminergic function (Howes, McCutcheon, & Stone, 2015). New compounds such as SEP-363856 have also emerged as potential treatments of schizophrenia by targeting presynaptic proteins such as the *trace amine-associated receptor 1* expressed in the dopaminergic neurons (Koblan et al., 2016).

Dopamine receptors are conventionally classified as D_1-like (D_1 and D_5) and D_2-like (D_{2S}, D_{2L}, D_3 and D_4) family receptors, where the D_1-family receptors are coupled with a G_s subunit, while the D_2-family receptors are coupled with a G_i subunit.

2.1.1. D_1

The D_1 receptors are mostly present on postsynaptic neurons, and are densely expressed in the striatum, amygdala, olfactory bulb, cerebellum, and PFC (Ariano & Sibley, 1994; Bergson et al., 1995). According to Santana and Artigas (2017), the D_1 receptors in the rat PFC are expressed on GABAergic interneurons and glutamatergic pyramidal neurons, with some preference for the first group. The PFC is implicated in cognitive processes such as reasoning, planning, and spatial ability (Wood & Grafman, 2003), and particularly for this reason the cognitive role of D_1 receptors in schizophrenia has been investigated in various imaging and pharmacological studies.

Using PET, Okubo et al. (1997) demonstrated that binding of a radioligand to the D_1 receptor was reduced in the PFC of schizophrenics, and that this reduction was related to the severity of the negative symptoms. On this subject, Aoyama et al. (2014) studying the psychostimulant phencyclidine (PCP) showed that clozapine was able to reverse the PCP-induced behavioral deficits in rats, through the activation of dopamine D_1 receptor signaling with an increase of histone H_3 acetylation. These findings indicate that the D_1 receptor in the PFC may have a role in clozapine action. However, this effect is most likely mediated by a dopamine increase in the PFC though clozapine antagonism on noradrenergic α_2 receptors (Devoto, Flore, Pira, Longu, & Gessa, 2004). Intriguingly, Chen and Yang (2002) demonstrated that clozapine, by increasing dopamine release and in turn activating the D_1 receptor, was able to induce NMDA-induced currents in cortical pyramidal cells. Similar to clozapine, asenapine, still a strong α_2 receptor antagonist, was shown to increase cortical dopaminergic and NMDA receptor-mediated transmission in rats.

Karlsson et al. (1995) confirmed the relevance of dopamine function in the PFC. They also showed that selective D_1 receptor antagonists are not only inefficacious in improving any symptoms in schizophrenia, but eventually may even exacerbate some aspects of this disorder.

2.1.2. D_2

In the central nervous system (CNS), D_2 receptors are mostly expressed in the striatum, the nucleus accumbens (NAc), and the olfactory tubercle. In addition, they are also present in the substantia nigra, ventral tegmental area (VTA), hypothalamus, cortical areas, septum, amygdala, and hippocampus (Missale, Nash, Robinson, Jaber, & Caron, 1998; Seeman, Wilson, Gmeiner, & Kapur, 2006; Vallone, Picetti, & Borrelli, 2000). Functionally, the D_2 receptor is classically assumed to signal through $G_{i/o}$ and other proteins, such as β-arrestin (Quan, Kim, Albert, Choi, & Kim, 2008).

Neuroimaging analyses have established that the optimal D_2 receptor occupancy for TAPs is between 65 to 80% in the striatum, where extrapyramidal symptoms (EPS) may occur when more than 80% of D_2 receptors are blocked (Uchida et al., 2011). In contrast, the optimal therapeutic window of D_2 receptor occupancy for AAPs is not stringent because AAPs can regulate dopamine hyperactivity through alternative mechanisms besides D_2 receptor antagonism. However, if an AAP reaches a receptor occupancy of 80% or above, EPS are likely to occur. This is relevant especially for risperidone, and eventually for olanzapine, as they have high affinity for the D_2 receptor and at certain dosages can have a receptor occupancy of 80% or above (Fig. 1). On the contrary, clozapine and quetiapine never show a D_2 receptor occupancy above 80% at their therapeutic concentrations, which could explain why they never cause parkinsonism.

Another elegant pharmacological approach to reduce the risk of EPS is by using partial agonists at the D_2 receptor, as demonstrated by aripiprazole. This compound behaves as an antagonist when dopamine is in excess, but intrinsically is still able to partially activate the D_2 receptor up to 20-40% (Yokoi et al., 2002). Thus aripiprazole has a dual agonist/antagonist action depending on synaptic levels of dopamine. Hence, it has a lower incidence of EPS, though at higher doses this undesired side effect might appear.

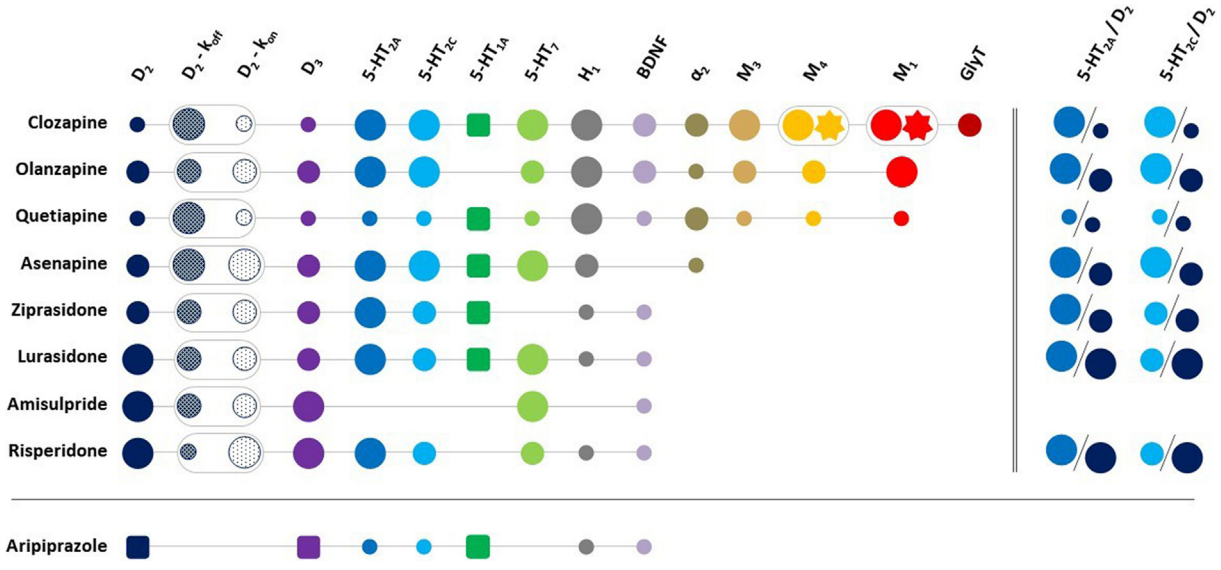

Fig. 1. Molecular targets of AAPs. List of the most relevant targets involved in the mechanism of action of AAPs based on receptor occupancy. Values are reported as high (●), medium (●) and low (●). ●, ■ and ✹ represent receptor antagonism, partial agonism and positive allosterism, respectively. ⊗ and ○ represent k_{off} and k_{on} values for the D_2 receptor and ● represents BDNF production, while ✹ represents positive allosterism by the clozapine metabolite, norclozapine, at M_1 and M_4 receptors. Aripiprazole is shown at the bottom for its different mechanism of action. Finally, the 5-HT_{2A}/D_2 and 5-HT_{2C}/D_2 receptor affinity ratios are included on the right. Clozapine covers a wide-range of molecular targets among all AAPs, while risperidone and amisulpride are mostly limited to just a few, and this might explain clozapine's superiority among AAPs.

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2.1.3. D_2 k_{off} and k_{on}

Beyond D_2 receptor affinity, one of the most enduring and sophisticated hypotheses on the mechanism of AAPs is based on their fast dissociation kinetic (k_{off}) from dopamine D_2 receptors (Kapur & Seeman, 2001). These particular kinetic properties were introduced to explain the lower incidence of side effects such as parkinsonism and hyperprolactinemia in AAPs compared to TAPs. APs are competitive antagonists that form reversible bonds with the D_2 receptor. At equilibrium, the amount of drug bound to the D_2 receptor is constant, but receptor occupancy is dynamically determined based on how fast the drug-receptor complex is displaced in response to changes in the dopamine levels in the synaptic cleft.

Specifically, fast dissociation of the AP allows a greater fraction of the D_2 receptor to be bound to transiently released dopamine in a surmountable manner, while drugs with slow k_{off} tend to prevent dopamine binding and practically behave as insurmountable antagonists. For example, AAPs such as clozapine and quetiapine show a very quick displacement from the D_2 receptor (Fig. 1) with a half-life in the order of seconds. However, the supposed linear correlation between fast dissociation and reduced side effects of AAPs is more complex than anticipated, particularly for risperidone, olanzapine and amisulpride, whose clinical characteristics go beyond their D_2 receptor k_{off} (Fig. 1) (Meltzer, 2013). To explain such complexity, first of all we need to underline the large experimental variability among the kinetic parameters determined with different methods, and secondly the assumption that AAPs have similar association rates (k_{on}) as previously proposed (Sahlholm et al., 2016).

Using time-resolved fluorescence resonance energy transfer assay analysis, Sykes et al. (2017) found very diverse values of both k_{on} and k_{off} among APs, demonstrating not only the importance of k_{off}, but also of k_{on} for determining D_2 receptor affinities (Fig. 1). Interestingly, they proposed that the drug-induced EPS are better associated with the k_{on}, while the k_{off} values seemed to be more related to the increase in prolactin secretion. For instance, they found a very slow k_{off} for risperidone, even lower than that for haloperidol, which may explain why hyperprolactinemia is so frequent with risperidone. They also found a high value of k_{on} for risperidone and the TAP chlorpromazine, which may be responsible for the incidence of parkinsonism.

In conclusion, based on the model presented by Sykes et al. (2017), we can assume that the AP-induced hyperprolactinemia is strongly correlated with D_2 receptor k_{off}, while AP-induced EPS depend on both receptor k_{off} and k_{on}, together with the contribution of other receptors (e.g. 5-$HT_{2A/2C}$) that may regulate dopamine release.

2.1.4. D_3

In the human CNS, the dopamine D_3 receptors seem to be less expressed than the two principal dopamine receptors, but more concentrated in certain areas. The D_3 receptors are expressed both as autoreceptors on dopaminergic neurons and as postsynaptic receptors (Diaz et al., 1995; Lévesque et al., 1992). Their highest expression is in the limbic areas, which are associated with emotional and cognitive functions, more specifically in the islands of Calleja, NAc and olfactory tubercle (Gurevich, Himes, & Joyce, 1999; Hurley & Jenner, 2006; Sokoloff & Le Foll, 2017).

AAPs bind to D_3 receptors with an affinity similar to that of D_2, so it is not easy to understand the specific contribution of the D_3 receptor subtype. However, selective D_3 antagonists seem to enhance dopaminergic neurotransmission, especially in the PFC where dopamine release is also controlled in part by D_3 autoreceptors (Lahti, Weiler, Carlsson, & Tamminga, 1998). The blockade of D_3 receptors enhances acetylcholine (ACh) release in the PFC that could contribute to pro-cognitive actions (Gobert et al., 1995; Lacroix et al., 2006). As a consequence, D_3 receptor antagonists are able to reverse the hyperactivity and social interaction deficits caused by NMDA receptor blockade in an animal model of schizophrenia (Sokoloff & Le Foll, 2017; Sokoloff, Leriche, Diaz, Louvel, & Pumain, 2013). Recently, the D_3 receptor was also shown to play a role in the neuroplasticity induced by the NMDA antagonist ketamine (Cavalleri et al., 2018).

Several selective D_3 receptor antagonists (i.e., SB277011A, S33084, GSK598809, F17464) were demonstrated to be efficacious in animal models of schizophrenia, and also in humans by improving attention, socialization and cognitive performance without causing EPS (Millan et al., 2007; Nakajimaa et al., 2013; Watson, Marsden, Millan, & Fone1, K. C. F., 2012). In a phase II trial involving patients with acute exacerbation of schizophrenia, the selective ligand F17464 demonstrated superiority over placebo, along with a good safety profile (Bitter et al., 2017). In addition, two AAPs currently used in therapy are blonanserin, a D_3 receptor preferring antagonist and caripiprazine, a D_3 receptor partial agonist (Baba, Enomoto, Horisawa, Hashimoto, & Ono, 2015; Girgis et al., 2016). Cariprazine reduced both positive and negative symptoms in schizophrenic patients, and particularly with regard to negative symptoms it performed better than risperidone (Garnock-Jones, 2017). The improvement on cognitive tasks was mediated by D_3 receptors, as demonstrated in knockout mice models (Zimnisky et al., 2013).

2.1.5. D_4

The dopamine D_4 receptors are mainly distributed in the PFC, entorhinal cortex, and hippocampus, regions particularly important for cognition, with a less significant distribution on the medium spiny neurons in the striatum and thalamus of rodents and humans (Lauzon & Laviolette, 2010; Rondou, Haegeman, & Van Craenenbroeck, 2010; Thomas, Grandy, Gerhardt, & Glaser, 2009). Initially, some authors hypothesized a possible role of excessive D_4 receptor stimulation in the pathophysiology of schizophrenia because a high density of D_4 receptors has been found in the brains of schizophrenics, and clozapine has a high affinity for this receptor (Seeman, Guan, & Van Tol, 1993; Van Tol et al., 1991). However, the upregulation of D_4 receptors in postmortem brains of schizophrenic patients was not confirmed by other studies (Hwang et al., 2012; Tarazi, Yeghiayan, Neumeyer, & Baldessarini, 1998).

A considerable number of preclinical and clinical studies has been carried out to investigate the role of D_4 receptors in schizophrenia, but none of the selective antagonists, such as L745870 and sonepiprazole, improved any condition of schizophrenia. In addition, it has been shown that activation of D_4 receptors in the PFC elevates cortical ACh and dopamine efflux, which could significantly contribute to pro-cognitive effects (Woolley et al., 2008). Recently, Cardozo et al. (2017) employed an innovative in silico approach and demonstrated that the D_4 receptors in the pineal gland can be a unique target for clozapine compared to chlorpromazine. The pineal gland produces melatonin and thus strongly influences mood via circadian rhythms. Also, González, Moreno-Delgado et al. (2012) proposed that the production of both melatonin and serotonin is perhaps regulated by the heteromerization of noradrenergic and dopamine D_4 receptors, which represent a key functional unit able to modify the circadian rhythm.

2.2. Serotoninergic (5-HT) system and serotonin receptors

Serotonin, via its many receptors (15 5-HTRs), is capable of having a profound impact on dopaminergic, glutamatergic and GABAergic neurons and other neurotransmitters in the human brain (Fig. 2). In addition to an overactivity of the glutamatergic system in the medial prefrontal cortex (mPFC) as a pathophysiological marker of schizophrenia, hyperactive serotoninergic transmission has also been proposed to be involved, and clozapine and not haloperidol was able to stabilize the serotonin increase in the mPFC in the MK-801-based animal model of schizophrenia (López-Gil et al., 2007).

Besides the hypothesized 5-HT_{2A}/D_2 ratio-based mechanism of AAPs, other serotonin receptors have also been considered as potential targets of different AAPs (Fig. 1). In fact, today it is clear that receptors such as 5-HT_{2C} and 5-HT_{1A} have an important role similar to that of 5-HT_{2A} in the mechanism of action of AAPs. In addition, other serotonin

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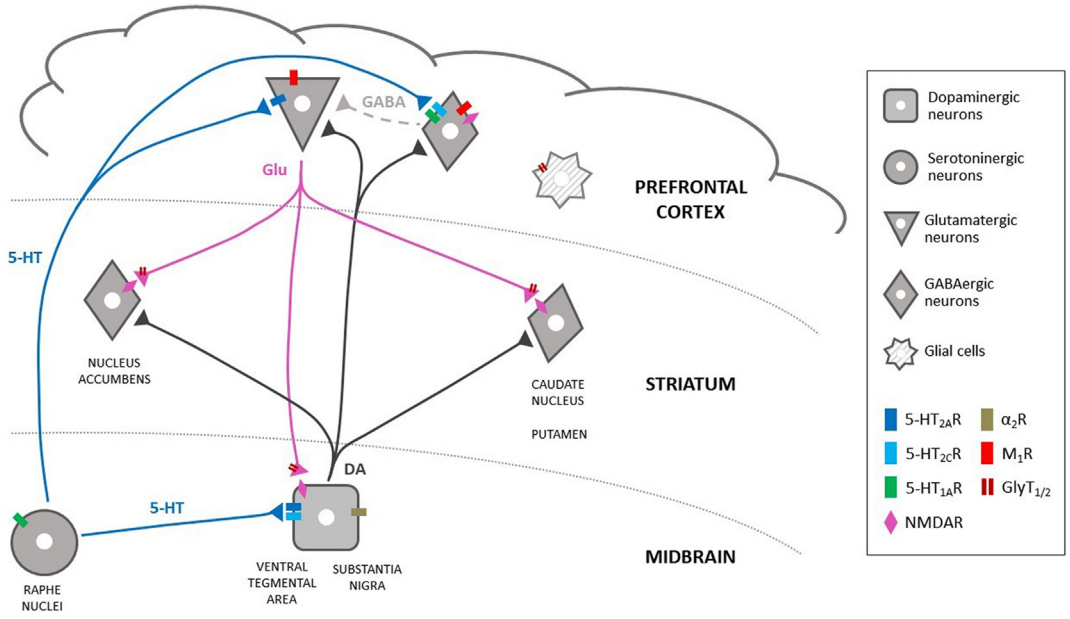

Fig. 2. Schematic representation of the main neurotransmitter pathways and receptors regulating the dopaminergic system. Dopaminergic neurons of Ventral Tegmental Area and Substantia Nigra project toward the Striatum (Nucleus Accumbens, Caudate Nucleus and Putamen) and the Prefrontal Cortex. Serotoninergic neurons (Raphe Nuclei) and glutamatergic neurons (Prefrontal Cortex) profoundly influence the dopaminergic activity via direct and indirect pathways. The GABAergic interneurons and glial cells are also illustrated. Serotoninergic (5-HT_{2A}R, 5-HT_{2C}R and 5-HT_{1A}R), muscarinic (M_1R), noradrenergic (α_2R) and glutamatergic (NMDAR) receptors as well as glycine transporters ($GlyT_{1/2}$) are expressed in different neuronal populations, and they are relevant to explain the differences in the mechanism of action of AAPs.

receptors such as 5-HT_6 and 5-HT_7 have started to receive attention as potential new targets for some AAPs such as amisulpride and others. The general idea is that the enhanced dopamine efflux that is mediated by blockade of 5-HT_2 receptors compensates for the AP effect of blocking dopamine receptors, thereby dampening the deleterious effect associated with the blockade of D_2 receptors (Kapur & Remington, 1996; Saller, Kreamer, Adamovage, & Salama, 1989). As a confirmation regarding the relevance of 5-HT_2 receptors in schizophrenia and other psychoses, the administration of the 5-HT_{2A} agonist lysergic acid diethylamide in mice is a well-known model of schizophrenia with behaviors such as hyperactivity and decreased social interaction.

2.2.1. 5-HT$_{1A}$

The 5-HT_{1A} receptors have complex multiple functions in the CNS and are expressed in different areas of the brain, mainly in the cortex, hippocampus, amygdala and VTA. In addition, they are also located in the raphe nuclei where they act as serotonin autoreceptors (Hall et al., 1997; Pompeiano, Palacios, & Mengod, 1992). In the cortex, they are localized on glutamatergic pyramidal cells and GABAergic interneurons, and they can be co-localized with 5-HT_{2A} and 5-HT_{2C} receptors (Amargós-Bosch et al., 2004; Santana, Bortolozzi, Serrats, Mengod, & Artigas, 2004). The 5-HT_{1A} receptors are inhibitory and coupled to G_i protein.

5-HT_{1A} receptor agonists, such as 8-OH-DPAT, increase dopamine efflux in the PFC and hippocampus (Sakaue et al., 2000), an effect that seems related to an inhibitory action on GABAergic interneurons. In fact, this action results in disinhibition of glutamatergic pyramidal neurons, which enhances dopaminergic neuronal activity and increases dopamine release (Fig. 2). This mechanism might also be responsible for an increased release of ACh in the PFC and hippocampus, which could potentially improve cognitive functions. Many AAPs such as clozapine, quetiapine, aripiprazole and ziprasidone are 5-HT_{1A} receptor partial agonists, which may be relevant for their mechanism of action (Fig. 1). Intriguingly, the selective 5-HT_{1A} receptor antagonist WAY 100635 is able to reduce cortical dopamine release induced by AAPs that do not have an affinity for the 5-HT_{1A} receptor, implying an indirect role of this receptor in the mechanism of AAPs. Based on this premise, the 5-HT_{1A} receptor partial agonist buspirone, when used together with haloperidol in schizophrenic patients, resulted in a beneficial effect on psychotic and cognitive symptoms and parkinsonism (Meltzer & Sumiyoshi, 2008; Sovner & Parnell-Sovner, 1989). Novel compounds with D_2 receptor antagonistic and 5-HT_{1A} receptor agonistic properties, such as SLV-313, SSR-181507, F-15063, S-16924, BSF 190555 (BTS 79018) and RGH-188, have been synthetized as new AAP candidates (Birch, Bradley, Gill, Kerrigan, & Needham, 1999; Claustre et al., 2003; De Berardis et al., 2016; McCreary et al., 2007; Millan et al., 1999; Newman-Tancredi, 2010). It should not be neglected, however, that the effect of clozapine to eliminate MK-801-induced hyperactivity was left unchanged in 5HT_{1A}-receptor knockout mice, leading to the conclusion that the 5-HT_{1A} receptors are not mandatory at least for this specific activity of clozapine.

2.2.2. 5-HT$_{2A}$

The 5-HT_{2A} receptors are densely present in cortical regions, including the PFC and insular cortex (Doherty & Pickel, 2000; López-Giménez, Mengod, Palacios, & Vilaró, 1997; Wright, Seroogy, Lundgren, Davis, & Jennes, 1995). At the cellular level, they are expressed by both glutamatergic pyramidal cells and GABAergic interneurons. The 5-HT_{2A} receptors are GPCRs coupled to the G_q and PI-PLC pathway. Early studies regarding the role of 5-HT_{2A} receptors in regulating dopaminergic neuronal activity and dopamine release were carried out with drugs that were unable to discriminate different 5-HT_2 receptor subtypes, thereby giving rise to misleading conjectures. Subsequently, many functions attributed to the 5-HT_{2A} receptors have been reconsidered and assigned to other receptors like 5-HT_{2C}. In general, the 5-HT_{2A} receptors can facilitate dopamine efflux in all regions of the brain with an excitatory function. However, the modulation on different regions can be complex as an inhibitory activity was also found in some cases, particularly in the PFC. The 5-HT_{2A} receptor-dependent control of dopamine release in the cortex is similar to that of 5-HT_{1A} receptors and involves a long glutamatergic loop (Fig. 2).

Recently, a correlation was found amongst the clinical doses of some AAPs, and the D_2 versus $5\text{-HT}_{2A}/5\text{-HT}_{1A}$ or the D_2 versus $5\text{-HT}_{2C}/5\text{-HT}_{1A}$ affinity ratios were used to suggest the relevance of high affinity towards 5-HT_{2A}, 5-HT_{2C} and D_2 receptors (Łukasiewicz et al., 2010).

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The blockade of 5-HT_{2A} receptors has a strong impact on dopaminergic activity, leading to reduction of dopamine release in the mesolimbic areas that contributes to AAP activity. Nevertheless, we cannot exclude an activity of specific GABAergic interneurons in the cortical areas, where 5-HT_{2A} receptor antagonism on specific subpopulations of dopaminergic neurons can increase dopamine release.

In humans, pimavanserin, a preferential 5-HT_{2A} receptor antagonist/inverse agonist, was well tolerated when tried as augmentation strategy for treating schizophrenia, and moreover, it potentiated the therapeutic effects of low-dose risperidone (Abbas & Roth, 2008). On the other side, when the selective 5-HT_{2A} receptor antagonist SR43469B was tested as a monotherapy to treat the acute phase of schizophrenia, it was found to be less effective than haloperidol, but better than placebo (Meltzer, Arvanitis, Bauer, & Rein, 2004). Strikingly, pimavanserin in monotherapy has proved to be effective in reducing psychoses in Parkinson's disease (PD), providing significant evidence for the relevance of 5-HT_{2A} and most likely also 5-HT_{2C} receptors in psychotic symptoms (Meltzer et al., 2010).

2.2.3. 5-HT_{2C}

The 5-HT_{2C} receptors are widely distributed in the brain, particularly in cortical areas including the PFC, in limbic structures including the hippocampus, in the striatum (NAc), in the mesencephalon and in the choroid plexus. This receptor subtype is often expressed by inhibitory GABAergic interneurons (Fig. 2). The 5-HT_{2C} receptors are GPCRs coupled to the G_q and PI pathway, and they mainly exert an inhibitory control on all ascending dopamine pathways, although excitatory effects have also been reported, thereby confirming the complexity of 5-HT_{2C} receptor action on different neuronal subpopulations.

A constitutive activity of 5-HT_{2C} receptors may be responsible for inhibition of dopaminergic neuron activity (Fig. 2), with relevant applications to the pharmacology of inverse agonists. Notably, clozapine and other AAPs behave as 5-HT_{2C} receptor inverse agonists in vivo, and this could be relevant to the clinical outcomes of these agents (Herrick-Davis, Grinde, & Teitler, 2000; Navailles, De Deurwaerdère, & Spampinato, 2006; Rauser, Savage, Meltzer, & Roth, 2001). In general, 5-HT_{2C} receptor agonists reduce dopamine release in the cortex, striatum and NAc, while 5-HT_{2C} receptor antagonists have the opposite effect (De Deurwaerdère, Navailles, Berg, Clarke, & Spampinato, 2004; Meltzer & Huang, 2008). Some studies have pointed out that the severity of EPS may be inversely correlated to the affinity of the AAPs to 5-HT_{2C} receptors (Gunes, Dahl, Spina, & Scordo, 2008; Richtand et al., 2007). Based on the evidence that 5-HT_{2C} receptor stimulation inhibits the mesolimbic dopaminergic system, 5-HT_{2C} receptor agonism could in theory have a therapeutic potential in improving the positive symptoms of schizophrenia (Alex, Yavanian, McFarlane, Pluto, & Pehek, 2005; Marquis et al., 2007; Meltzer, 1999; Pozzi, Acconcia, Ceglia, Invernizzi, & Samanin, 2002).

Moreover, 5-HT_{2C} receptors seem to be important for cognition as they are able to modulate not only dopamine, but also ACh, particularly in the hippocampus (Zhelyazkova-Savova, Giovannini, & Pepeu, 1999). In addition, blockade of 5-HT_{2C} receptors with a consequent increase of dopaminergic activity might exert some antidepressive activity, which contributes to the mechanism of action of atypical antidepressants like mirtazapine, trazodone and nefadozone (Di Matteo, De Blasi, Di Giulio, & Esposito, 2001; Giorgetti & Tecott, 2004; Guardiola-Lemaitre et al., 2014; Millan et al., 2000; Millan, Dekeyne, & Gobert, 1998).

2.2.4. 5-$HT_{6/7}$

The 5-HT_6 receptors are expressed at a higher level in the striatum, olfactory tubercle and NAc, and at a lower level in the cerebral cortex. Some studies have indicated that 5-HT_6 receptor antagonists may increase dopamine extracellularly in the mPFC (Lacroix, Dawson, Hagan, & Heidbreder, 2004) or hippocampus (Li, Huang, Prus, Dai, & Meltzer, 2007), though others could not confirm this evidence (Dawson & Li, 2003; Dawson, Nguyen, & Li, 2003; Li et al., 2007). Moreover, studies with 5-HT_6 receptor agonists were unable to reveal any conclusive information regarding the role of 5-HT_6 receptors in the control of dopamine release. However, the use of 5-HT_6 receptor antagonists in combination with D_2 receptor antagonists such as haloperidol potentiated dopamine release in the mPFC and hippocampus, suggesting a synergism between these two receptors (Li et al., 2007). Importantly, the administration of 5-HT_6 receptor antagonists reduced the effects of MK-801 and PCP in an animal model of schizophrenia, demonstrating the therapeutic potential of this receptor (de Bruin et al., 2013). Some AAPs like clozapine, olanzapine, asenapine and sertindole are potent 5-HT_6 receptor antagonists with different affinities (Abbas et al., 2009; Tarazi, Moran-Gates, Wong, Henry, & Shahid, 2010), and this may have some role in their actions.

The highest levels of 5-HT_7 receptors are found in the hypothalamus, thalamus, mesencephalon and hippocampus, while lower levels are present in the cerebral cortex. So far, the lack of selective ligands has made it difficult to identify a specific functional role of the 5-HT_7 receptor in dopaminergic activity. Similar to 5-HT_6, some AAPs like amisulpride, asenapine, clozapine, lurasidone and risperidone have high affinity for the 5-HT_7 receptor, which may contribute to their therapeutic actions (Fig. 1) (Roth et al., 1994). In particular, the 5-HT_7 receptor affinity of amisulpride shows that it may not simply be a dopaminergic compound, but also a serotoninergic one. This affinity for 5-HT_7 receptors endows amisulpride with the ability to improve novel object recognition in mice treated with PCP (Horiguchi, Huang, & Meltzer, 2011).

In conclusion, recent data underscore the relevance of 5-HT_6 and 5-HT_7 receptors as a component in the mechanism of some AAPs. Their actual relevance however is still a matter of debate, requiring more data for relevant conclusions.

2.3. Glutamatergic system and glutamate receptors

The glutamatergic system has a prominent role in the pathogenesis of schizophrenia and other psychoses, a fact confirmed by genetic studies that found mutated genes can dysregulate this system (Fournier et al., 2017). In fact, in drug-resistant schizophrenic patients or in patients with very limited response, abnormalities in the glutamatergic system may be particularly relevant (Howes et al., 2015; Jauhar et al., 2018; Mouchlianitis et al., 2016). Importantly, in non-responders, only clozapine seemed to be effective, at least to a certain degree, while in select few cases olanzapine showed partial efficacy at higher doses (Kannan et al., 2017). This evidence suggests that the superiority of clozapine is partially explained by its activity on the glutamatergic system. It is also true that the glutamatergic, dopaminergic and serotoninergic systems are strongly interconnected, which further complicates the analysis of the mechanism of action of AAPs on the glutamatergic system (Fig. 2).

Some studies in humans have suggested that increased glutamate efflux observed in the PFC and anterior cingulate cortex may be responsible for cognitive and negative symptoms of schizophrenia (Merritt, Egerton, Kempton, Taylor, & McGuire, 2016; Poels et al., 2014). Additionally, the use of NMDA receptor antagonists, such as PCP or MK-801, in mice reproduces aspects and behaviors that reflect human schizophrenic pathology. In studies involving mice and rats exposed to PCP, clozapine and other AAPs significantly attenuated the increased glutamate efflux observed in the PFC, followed by a reduction of impaired behavior (Dissanayake, Zachariou, Marsden, & Mason, 2009; Kargieman, Riga, Artigas, & Celada, 2012). In humans, few studies have reported a relation between the extent of glutamatergic reduction and symptomatic improvement over the AAP treatment period (Egerton et al., 2017; van der Heijden et al., 2004; Tascedda et al., 2001).

Noteworthy, the activity of serotonin 5-HT_{2A} and 5-HT_{1A} receptors seems to be relevant for controlling the glutamatergic system, and this may explain the superiority of AAPs over TAPs. In addition, effects on

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NMDA receptors and glycine transporters (GlyTs) that contribute to homeostasis in the glutamatergic system seem important for controlling glutamate hyperactivity, a feature which may be relevant particularly for clozapine's action.

2.3.1. NMDA

The NMDA receptor hypofunction has been intriguingly associated with schizophrenia aetiopathogenesis (Kannan et al., 2017). Besides glutamate, the NMDA receptor complex is modulated by other factors such as the amino acids glycine and D-serine that are produced endogenously, and by glutathione which regulates the redox-sensitive site (Balu & Coyle, 2015). Electrophysiological and behavioral studies have shown that AAPs such as clozapine, olanzapine and risperidone enhance NMDA receptor-mediated transmission, behaving as partial agonists at the glycine recognition site (Arvanov, Liang, Schwartz, Grossman, & Wang, 1997; Kargieman, Santana, Mengod, Celada, & Artigas, 2007; Ninan, Jardemark, & Wang, 2003). Hence, a direct action of AAPs at glycine sites has been speculated, but this hypothesis is yet to be experimentally confirmed (Millan, 2002; Schwieler, Linderholm, Nilsson-Todd, Erhardt, & Engberg, 2008).

Other mechanisms may be involved in how clozapine enhances the functional activity of the NMDA receptor, such as its phosphorylation by protein kinase A, which is possibly mediated by dopamine release that subsequently activates the D_1 receptor (Chen & Yang, 2002; Leveque et al., 2000; Tseng & O'Donnell, 2004). In addition, the clozapine metabolite N-desmethylclozapine (norclozapine), an M_1 receptor allosteric agonist, has been shown to potentiate NMDA receptor currents (Sur et al., 2003).

Indeed, agonists at the glycine site, like D-cycloserine and D-serine, have been found to potentiate the ability of some AAPs and TAPs to improve negative and positive symptoms in schizophrenics (Moghaddam & Javitt, 2012). In contrast, this additional effect was not observed with clozapine, indicating that clozapine itself is capable of enhancing NMDA receptor activity (Millan, 2005), most likely by increasing the release of glycine and/or D-serine from glial and neuronal cells through inhibition of different neutral amino acid transporters (Javitt, Duncan, Balla, & Sershen, 2005; Tanahashi, Yamamura, Nakagawa, Motomura, & Okada, 2012; Williams, Mallorga, Conn, Pettibone, & Sur, 2004). When tested in monotherapy or adjunct therapy, suppression of D-serine degradation by D-amino acid oxidase inhibitors such as sodium benzoate improved neurocognition, specifically speed of processing, visual learning and memory (Lane et al., 2013; Lin et al., 2017).

2.3.2. Glycine and neutral amino acid transporters

Among the AAPs, clozapine is the most effective in inhibiting GlyT in glial cells, and this effect mostly seems to involve GlyT1a compared to GlyT2 (Figs. 1, 2) (Williams et al., 2004). In addition, clozapine is able to enhance glycine levels by inhibiting sodium-coupled neutral amino acid transporter 1, and eventually sodium-coupled neutral amino acid transporter 2 sites, on neuronal cells (Javitt et al., 2004; Schwieler, Engberg, & Erhardt, 2004), the so called system A-mediated GlyT (Javitt et al., 2005). A relationship between GlyT1 inhibition and improvement of cognitive performance, such as working memory in primates treated with ketamine, has been found using PET techniques, where the blockade of this transporter was more than 75%. Importantly, sarcosine, an inhibitor of glycine transport, has been tested in clinical trials either as monotherapy or in association with AAPs, in which it has shown some promising results (Lane et al., 2008). In a recent phase 2 clinical study, a new GlyT1 inhibitor RG1678 (bitopertin) was found to be effective in schizophrenic patients with predominant negative symptoms (Pinard et al., 2010). However, this compound failed in several phase 3 clinical trials when studied in patients with persistent negative symptoms and residual positive symptoms (Bugarski-Kirola et al., 2016, 2017; Goff, 2014).

2.4. Cholinergic system and muscarinic receptors

The two major groups of cholinergic projections are the pedunculopontine cholinergic complex, which projects to various midbrain and brainstem structures, and the basal forebrain complex, which originates in the nucleus basalis of Meynert and projects to the hippocampus and to cortical regions (Henny & Jones, 2008). The muscarinic receptor family consists of five subtypes, M_1 to M_5, which are expressed throughout the brain and play a role in a wide range of functional processes, such as learning, memory, attention, sensorimotor processing, sleep-wake cycles and arousal (Conn, Jones, & Lindsley, 2009; Wess, Eglen, & Gautam, 2007).

The M_1, M_3, and M_5 receptors classically signal through $G_{q/11}$, and mediate the excitatory neuromodulatory actions of ACh, whereas the M_2 and M_4 receptors signal through $G_{i/o}$ and mediate the inhibitory neuromodulatory actions of ACh (Felder, 1995).

Furthermore, by using mRNA techniques, specific antibodies and radioligand binding assays, the M_1 receptor was found to be the major postsynaptic receptor across the cholinergic, glutamatergic, and GABAergic neurons, whereas the M_2 and M_4 are the major presynaptic receptors at the cholinergic, glutamatergic and GABAergic synapse types in the brain (Lebois, Thorn, Edgerton, Popiolek, & Xi, 2017).

As shown by a neuroimaging study using single-photon emission computed tomography, the non-selective muscarinic receptor ligand [123I]-iodoquinuclidinyl benzilate decreased the expression of muscarinic receptors in the cortex and basal ganglia of schizophrenic patients compared to healthy subjects. More importantly, the severity of positive symptoms in these patients negatively correlated with expression levels, implying a relevance of muscarinic receptors in the pathophysiology of this neurological disorder (Raedler et al., 2003).

2.4.1. M_1

The M_1 receptors are abundantly expressed on glutamatergic neurons and GABAergic interneurons of the cortex (Fig. 2). Decreased M_1 receptor signaling has been linked to cognitive impairment associated with schizophrenia (CIAS) and thus, enhancement of M_1 receptor signaling has been postulated to be a therapeutic target for CIAS (Carruthers, Gurvich, & Rossell, 2015; Meltzer, 2015). In particular, M_1 receptor activation has been shown to induce depolarization of hippocampal CA1 pyramidal neurons, which increases glutamatergic neurotransmission that eventually leads to long-term-potentiation-mediated learning and memory formation (Dennis et al., 2016).

Clozapine was the first AAP reported to improve CIAS in schizophrenia (Hagger et al., 1993). Notably, even though clozapine is an antagonist at M_1, M_3, and M_5 receptors (Chew et al., 2008), its principal metabolite norclozapine behaves as positive allosteric modulator of the M_1 receptor (Fig. 1) (Sur et al., 2003; Yohn & Conn, 2017). Therefore, patients treated with clozapine who showed high norclozapine/clozapine ratios, also showed improved memory and reduced learning impairment as predicted by agonist/antagonist mixing studies (Bräuner-Osborne & Brann, 1996; Rajji et al., 2015). Moreover, direct stimulation of the M_1 receptor by NDMC and the M_1 receptor agonist xanomeline in rats promotes release of ACh and dopamine in the PFC and hippocampus, areas of the brain that are well known for its involvement in learning and memory (Li, Snigdha, Roseman, Dai, & Meltzer, 2008). Recently, Cardozo et al. (2017) showed that the M_1 receptor at the PFC is a specific and unique signature for clozapine's atypia.

In addition, although clozapine therapy usually lacks the traditional anti-cholinergic side effects like dry mouth, it could instead promote sialorrhea (Baldessarini & Frankenburg, 1991). These data strongly suggest that the enhanced M_1 receptor activity mediated by norclozapine is likely responsible for the improvements observed in memory and learning of schizophrenic patients, and regrettably for side effects like hypersalivation (Bymaster et al., 2003). Nevertheless, compared to potent classical muscarinic receptor antagonists, such as atropine and scopolamine, olanzapine has only a partial anti-

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muscarinic activity and therefore is better tolerated by patients (Bymaster et al., 1999).

2.4.2. M_4

Clinical studies with xanomeline in patients with schizophrenia suggest that the activation of M_1/M_4 receptors is effective in treating positive, negative, and cognitive symptoms of this disorder (Bolbecker & Shekhar, 2012; Shekhar et al., 2008). Although it was generally believed that M_1, but not M_4, receptor activation is associated with enhanced cognitive function, recent preclinical work suggests that the M_4 receptor also has an important role in cognitive function (Galloway, Lebois, Shagarabi, Hernandez, & Manns, 2014). Dopamine release induced by amphetamine, or by the NMDA receptor antagonist PCP, was found to be elevated in the NAc of M_4 receptor knockout mice, suggesting a role for the M_4 receptor in preventing hyperexcitability in midbrain dopamine neurons (Tzavara et al., 2004). Furthermore, M_4 receptor knockout mice showed increased basal locomotor activity along with PPI deficits, highlighting the potential of M_4 receptors in treating psychosis (Gomeza et al., 1999; Koshimizu, Leiter, & Miyakawa, 2012).

Regarding AAPs, clozapine behaves as M_4 receptor antagonist in the rat striatum (Olianas, Maullu, & Onali, 1997), while its metabolite norclozapine behaves as M_4 receptor agonist in the human neocortex (Fig. 1) (Gigout, Wierschke, Dehnicke, & Deisz, 2015). Olanzapine appears to be a weak partial agonist at the M_4 receptor (Zeng, Le, & Richelson, 1997), but in some experimental conditions it behaves as an antagonist instead (Zhang & Bymaster, 1999).

In summary, clozapine has a unique activity on muscarinic receptors, and the positive allosteric modulation of M_1 receptors through its metabolite norclozapine is relevant for its cognitive effect and other peculiar characteristics. Importantly, olanzapine is a muscarinic receptor antagonist with a weak/medium affinity and this may be relevant to explain the low risk to cause EPS.

2.5. Histaminergic system and histamine receptors

Histamine is synthesized by histidine decarboxylase and acts on the four histamine receptors H_1, H_2, H_3 and H_4. The histaminergic projections in the CNS originate from the tuberomamillary nucleus of the posterior hypothalamus and innervate many regions of the brain, including the cerebral cortex, hippocampus, amygdala, striatum and other areas of the brain stem. The activity of histaminergic neurons is regulated by a wide variety of neurochemicals such as glutamate, glycine, GABA, biogenic amines, purines, peptides and metabolic signals (Haas, Sergeeva, & Selbach, 2008).

2.5.1. H_1

The H_1 receptor is ubiquitously expressed, specifically in the CNS and blood vessels, and has an excitatory activity preferentially coupling to $G_{q/11}$ proteins (Panula et al., 2015; Seifert et al., 2013). In the CNS, the H_1 receptor is involved in regulating locomotor activity, emotions, cognitive functions, arousal, sleep, circadian rhythm and pain perception. Moreover, the H_1 receptor participates in the modulation of energy consumption, food intake and respiration (Schneider, Neumann, & Seifert, 2014). Clozapine, olanzapine and quetiapine have high occupancy values for the H_1 receptors in human brain at minimum clinical doses (Sato et al., 2015). The H_1 receptor is found to be expressed in the superior cervical ganglion, and the action of clozapine at this level may be a factor partly responsible for its adverse effects, i.e. orthostatic hypotension and hypersalivation, the latter due to innervation of salivary glands from the superior cervical ganglion (Cardozo et al., 2017). Weight gain is one of the major side effects of AAPs like clozapine, olanzapine, quetiapine and asenapine, which have a very high affinity for H_1 receptors (Kim, Huang, Snowman, Teuscher, & Snyder, 2007; Kroeze et al., 2003). Instead, risperidone and ziprasidone have low to medium affinity for this receptor, and this might explain the reduced weight gain in patients treated with them. H_1 receptor antagonism is also responsible for sedation, a side effect that may be helpful in acute psychoses, particularly in agitated patients (Fig. 1) (Fang et al., 2016).

2.5.2. H_3

The H_3 receptor is mainly a presynaptic autoreceptor, and it acts as a presynaptic heteroreceptor on non-histaminergic neural systems. It inhibits the release of histamine and other neurotransmitters, such as ACh, noradrenaline, dopamine or glutamate (Haas et al., 2008). Some preclinical studies have highlighted the possible role of H_3 receptor antagonism in treating schizophrenia, particularly its role in cognition (Ito, 2009), which was confirmed in schizophrenic patients by a mild effect to improve cognition (Jarskog et al., 2015). In striatal postsynaptic GABAergic neurons, the H_3 and also the D_1 and D_2 receptors are colocalized and form heterodimers, and this crosstalk has made way for the possible development of new APs (Ferrada et al., 2009; Vohora & Bhowmik, 2012). AAPs acting on the H_3 auto- and heteroreceptor, such as clozapine, facilitate the release of histamine, noradrenaline, ACh and serotonin, and these neurochemical changes are partly responsible for increase in food intake and improved cognition (Deng, Weston-Green, & Huang, 2010).

2.6. Noradrenergic system and noradrenergic receptors

The main source of noradrenaline in the CNS is the locus coeruleus, a small cluster of neurons located in the pons of the brainstem. Their projections distribute broadly to the neocortex, hippocampus, thalamus, subthalamic nucleus and substantia nigra, and to a lesser extent to striatum and spinal cord (Delaville, Deurwaerdère, & Benazzouz, 2011). Noradrenaline acts on the noradrenergic α and β receptors. Generally, the α_1 and β receptors are stimulatory, while the α_2 receptors are inhibitory. The noradrenergic heteroreceptors are found on glutamatergic, GABAergic, dopaminergic, serotoninergic, histaminergic and orexinergic neurons, showing a broad role of this neurotransmitter in regulation of other neurotransmitter systems (Maletic, Eramo, Gwin, Offord, & Duffy, 2017). Many AAPs have an affinity for the noradrenergic α_1 and α_2 receptors as antagonists, and in spite of limited clinical evidence, a role for the noradrenergic system has been proposed in schizophrenia (Uys, Shahid, & Harvey, 2017).

2.6.1. α_1 and α_2

The noradrenergic α_1 receptors have a role in controlling the mesolimbocortical dopaminergic neurons, and in stimulating the locus coeruleus, which affects dopaminergic neurons of the VTA. Noradrenergic α_1 receptor antagonism by many AAPs, particularly clozapine, is believed to contribute to the control of positive symptoms, and to mediate the correct firing of dopaminergic mesolimbic neurons (Svensson, 2003). In an experiment involving rodents, the deficits induced via prepulse inhibition was reversed by clozapine, olanzapine and quetiapine, behaving as α_1 receptor antagonists (Carasso, Bakshi, & Geyer, 1998).

These results indicate that adding α_1 receptor blockade to D_2 receptor antagonism might synergistically contribute to overall AP activity. Clozapine and other AAPs could also indirectly act on the dopaminergic activity through 5-$HT_{2A/2C}$ and α_1 receptors, with a lower occupancy of D_2 receptors and minimal interference with the reward system (Svensson, 2003). Due to its role in energy regulation, the direct antagonism of α_1 receptors increases adipogenesis, decreases energy expenditure and increases body weight (Basile et al., 2001).

In contrast to most other APs, clozapine, and norquetiapine (main active metabolite of quetiapine) act as antagonists at the α_2 receptor, and this peculiar feature has been hypothesized to contribute to their clinical profile (Fig. 1). Risperidone has a much lower affinity for the α_2 receptor than clozapine, but a slightly higher affinity than other AAPs (Svensson, 2003). The α_2 receptor antagonism of clozapine and norquetiapine can be important for their antidepressive characteristics,

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and this effect could partly explain the superiority of clozapine in preventing suicide (Meltzer et al., 2003).

The α_2 receptor blockers modulate firing of dopamine neurons in the VTA (Fig. 2), and this may contribute to an increase of dopamine in the mPFC (Kuroki, Meltzer, & Ichikawa, 1999; Svensson, 2003), an effect that is correlated with the affinity for α_2 and 5-$HT_{2A/2C}$ receptors. Intriguingly, dopamine may also be released from noradrenergic neurons behaving as a cotransmitter (Devoto, Flore, Pani, & Gessa, 2001). Moderate D_2 receptor blockade with a strong α_2 receptor antagonist may be a good profile for an AP, and this could be another peculiarity of clozapine's mechanism of action (Svensson, 2003). The importance of α_2 receptors in schizophrenia has also been demonstrated by the improvement of Positive and Negative Syndrome Scale positive scores when mirtazapine was co-administered with TAPs (Terevnikov et al., 2010).

2.7. Neurotrophic factors, synaptogenesis and neurogenesis

Several neuronal markers of neuroplasticity, such as the brain-derived neurotrophic factor (BDNF), have been found altered in the brain and/or in the plasma of schizophrenic patients (Kim & Na, 2017; Miller, Buckley, Seabolt, Mellor, & Kirkpatrick, 2011; Molteni et al., 2009). Accordingly, several studies have considered the role of various neurotrophic factors in the mechanism of action of AAPs. Among them, nerve growth factor (NGF), fibroblast growth factor (FGF2) and especially BDNF have received most attention.

Several experiments in animal models representing pathological conditions of schizophrenia have shown that AAPs, but not the TAP haloperidol, were able to reverse reduction of hippocampal BDNF expression (Fumagalli et al., 2004). In normal animals, during acute treatment, AAPs have a limited effect on BDNF expression, and overall these results are contradictory. On the contrary, during chronic treatment, AAPs tend to increase BDNF expression, while haloperidol induces down-regulation (Angelucci, Mathé, & Aloe, 2000; Chlan-Fourney, Ashe, Nylen, Juorio, & Li, 2002; Lipska, Khaing, Weickert, & Weinberger, 2001). In this case, increased expression of BDNF is dependent on drug usage. For instance, olanzapine was able to increase BDNF expression at a lower dose, while it reduced the expression at a higher dose. A reduction of hippocampal BDNF mRNA was also observed at high doses of risperidone (Chlan-Fourney et al., 2002). Following 8 months treatment of drug-naïve first-episode schizophrenic patients, olanzapine, quetiapine, risperidone, aripiprazole and amisulpride significantly increased serum BDNF levels, and they were able to increase the volume of the left hippocampus (Rizos et al., 2014). Moreover, the chronic use of lurasidone in rats increased the total BDNF mRNA levels in the PFC, and to a lesser extent in the hippocampus (Fig. 1) (Fumagalli et al., 2012). Finally, ziprasidone significantly attenuated the decrease in BDNF mRNA expression in the hippocampus and neocortex induced by stress in rat models of schizophrenia (Park et al., 2009).

NGF is known to be relevant in the peripheral nervous system and for cognitive functions. Haloperidol-induced reduction of neurotrophins in vivo (rodent model) was counteracted by AAPs, and the NGF levels were restored by risperidone and clozapine, while the BDNF levels were increased by olanzapine (Parikh, Khan, & Mahadik, 2004; Parikh, Terry, Khan, & Mahadik, 2004). However, the role of NGF in the mechanism of action of AAPs has not been studied as much as that of BDNF (Molteni et al., 2009).

Other than the most studied neurotrophic factors BDNF and NGF, FGF2 may also have a role in the mechanism of action of APs. Moreover, FGF2 is ubiquitously expressed in the adult brain, with the highest expression in hippocampus and cortical areas (Turner, Watson, & Akil, 2012). Several studies involving haloperidol, chlorpromazine, clozapine, quetiapine and olanzapine found that the induction of FGF2 was unique to clozapine (Molteni et al., 2009). In a subsequent study (rodent model), chronic treatment with a combination of fluoxetine and olanzapine showed increased FGF2 mRNA levels in the PFC, as well as in hippocampus and striatum (Chertkow, Weinreb, Youdim, & Silver, 2009; Maragnoli, Fumagalli, Gennarelli, Racagni, & Riva, 2004), with a significant contribution in brain function and plasticity (Maragnoli et al., 2004). In a similar rodent model, quetiapine completely reversed the MK-801-mediated decrease in BDNF and FGF2 mRNA levels (Fumagalli et al., 2004).

Neurotrophins regulate signaling pathways which influence the activities of important kinases, like Akt, ERK and GSK3β (Huang & Reichardt, 2001). In an in vitro experiment, the AAPs olanzapine, quetiapine and clozapine, but not TAPs, increased the number of cells bearing neurites by enhancing Akt and ERK phosphorylation (Lu & Dwyer, 2005). Recently, our group demonstrated that clozapine and other AAPs behave as biased agonists and activate ERK phosphorylation in different cell lines through a 5-HT_{2A} receptor-mediated G protein independent pathway (Aringhieri et al., 2017). This evidence was preceded by another study on an animal model of schizophrenia, where the relevance of 5-HT_{2A}-receptor-mediated Akt activation was used to explain clozapine's AP activity (Schmid, Streicher, Meltzer, & Bohn, 2014).

Additionally, it was hypothesized that the AAPs may stimulate neurogenesis. Recently, in a rat model of stress-induced impairments in neuronal structure in the hippocampus and PFC regions, clozapine upregulated adult neurogenesis and neuronal survival, whereas haloperidol promoted a downregulation of these processes (Morais et al., 2017). Similar to clozapine, other AAPs like quetiapine, olanzapine and aripiprazole have also been shown to increase neural proliferation (Chikama et al., 2017).

In conclusion, there is a plethora of preclinical evidence suggesting that AAPs compared to TAPs offer a better profile in terms of neuro- and synaptogenesis, with increased expression of neurotrophic factors such as BDNF. The modulation of adult neuroplasticity promoted by AAPs may be relevant in the long-term treatment of schizophrenia.

3. New mechanisms of action of AAPs

3.1. Biased agonism at dopamine and serotonin receptors

According to the classical model for GPCR activation, agonist binding to the receptor leads to conformational changes within the receptor structure that results in the activation of the associated heterotrimeric G protein. Nonetheless, over the past decade new mechanisms associated with GPCR function have been discovered, such as the ability of β-arrestins to act as multifunctional proteins and to activate multiple mediators like ERK, proto-oncogene tyrosine-protein kinase SRC, nuclear factor-κB and phosphoinositide 3-kinase (Rajagopal, Rajagopal, & Lefkowitz, 2010). The capacity of a ligand to preferentially activate either G protein-dependent signaling or G protein-independent signaling is called “biased agonism” or “functional selectivity”. This innovative new concept reflects the heterogeneity and complexity of the different receptor conformation states it can be transitioning when specifically interacting with stimulants (Kenakin, 2013). In addition, recent data have demonstrated how receptor functional selectivity is a dynamic and adaptable process, which can also be modified by physiopathological conditions (Kaya et al., 2012).

Biased agonism has important implications for the design of therapeutic drugs that target specific receptor activities. Furthermore, this new concept may be relevant to explain pharmacological differences that were unnoticed till date among drugs whose clinical differences were inexplicable. For example, biased agonism has been shown to be important to explain differences among the β-receptor antagonists for cardioprotection (Wisler et al., 2007) and among the μ-opioid receptor agonists for managing pain (Raehal & Bohn, 2005; Schmid et al., 2017).

For the D_2 receptor, one of the main target of APs, dopamine represents the endogenous ligand, which is equally effective in activating both the G_i-mediated cAMP inhibition and the β-arrestin 2 signaling

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(Fig. 3a). Dopamine induced β-arrestin 2 activation has been shown by using knockout mice models. In particular, Gainetdinov, Premont, Bohn, Lefkowitz, and Caron (2004) observed that mice lacking β-arrestin 2 have a reduced response to amphetamine-induced hyperlocomotion and to apomorphine-induced behaviors. In addition, these studies demonstrated that the D_2-mediated β-arrestin 2 downstream signaling inhibits Akt, and thus increases the activation of GSK3 (Beaulieu, Del'guidice, Sotnikova, Lemasson, & Gainetdinov, 2011). In this pathway, a protein complex is formed with β-arrestin 2, Akt and protein phosphatase 2A, which promotes the dephosphorylation/inactivation of Akt. Consistent with this pathway, APs blocking D_2 receptor activity would also prevent D_2 receptor-dependent β-arrestin 2 signaling, leading to an increased phosphorylation of Akt as suggested by experiments involving rodent brain. Indeed, both AAPs and TAPs blocked β-arrestin 2 translocation induced by quinpirole, while only TAPs, and not clozapine or other AAPs, were able to fully antagonize $G_{i/o}$ signaling (Fig. 3b) (Masri et al., 2008). This might explain the pharmacological differences among these two classes of APs, however, the clinical consequences are yet to be determined.

Studies on biased agonists have further elucidated the role of β-arrestin signaling in AP treatments. In particular, Allen et al. (2011) designed aripiprazole-derived D_2 receptor β-arrestin biased ligands, namely UNC9975, UNC0006 and UNC9994, which showed AP activity in vivo, but with less side effects. These compounds have a partial agonist activity on β-arrestin 2 recruitment and are antagonists on G_i signaling (Fig. 3c). From these data, they proposed that β-arrestin biased agonism may offer protection against motor side effects. This mechanism however was not shared by other AAPs, like clozapine. Taken together, these results suggest that both G protein and β-arrestin signaling pathways are determinants in D_2 receptor function, and that AAPs can differently modulate these dual activities. In addition, these studies provide new avenues towards targeting D_2 receptors to treat schizophrenia (Peterson et al., 2015).

In addition to the D_2 receptor, the 5-HT_{2A} receptor represents a prominent target of AAPs and its dual activity on the G protein and β-arrestin pathways has also been extensively demonstrated. In this context, although clozapine is classically considered as an antagonist on 5-HT_{2A} receptors, it has a peculiar pharmacological property such as activating Akt signaling through this receptor in vitro and in vivo. Thus clozapine behaves as a 5-HT_{2A} receptor biased agonist via a G protein-independent pathway (Fig. 4). Strikingly, clozapine-mediated suppression of MK-801 and PCP-induced hyperlocomotion in mice was dependent on 5-HT_{2A}-induced Akt activation, thereby confirming the relevance of this process in the AP activity of clozapine (Schmid et al., 2014). In line with this study, our group demonstrated in different cell lines that clozapine, via a similar mechanism, was effective at inducing ERK1/2 phosphorylation with a potency in the low micromolar range. Subsequently, we carried out a systematic comparison between AAPs and TAPs in relation to ERK 1/2 and Akt activation and found that only quetiapine and olanzapine were partially active on ERK, while TAPs like haloperidol and sulpiride did not have any relevant effect. Similar differences between AAPs and TAPs were also found for Akt phosphorylation (Aringhieri et al., 2017).

As previously mentioned, kinases such as ERK1/2 and Akt have received particular attention for their relevance in synaptic plasticity, neurogenesis, neuroprotection and neural processes that may be implicated in schizophrenia, and that they may also contribute to the mechanism of actions of AAPs in the long term. These recent findings add a new mechanism of action that may be partly responsible for the processes involving 5-HT_{2A} receptors. This peculiarity might explain the superior efficacy of clozapine compared to other AAPs.

3.2. Receptor homomers and heteromers

Many data show that GPCRs, apart from being monomers, form homodimers, heterodimers and higher-order oligomers through transient interactions on the plasma cell membrane (Scarselli et al., 2016). This evidence was provided by new techniques based on single-molecule microscopy mostly analyzing the formation of homodimers, whereas there is hardly any high-resolution data available in relation to the heteromerization process so far. There are several reports demonstrating that the dimerization process occurs in the endoplasmic reticulum as well as at the plasma membrane (Herrick-Davis, Weaver, Grinde, & Mazurkiewicz, 2006). The functional relevance of this phenomenon is still under scrutiny, for which many have found possible explanations (Maggio, Rocchi, & Scarselli, 2013; Scarselli, Annibale, Gerace, & Radenovic, 2013). These receptor complexes are potential novel targets for developing better drugs that are more selective, more effective, and

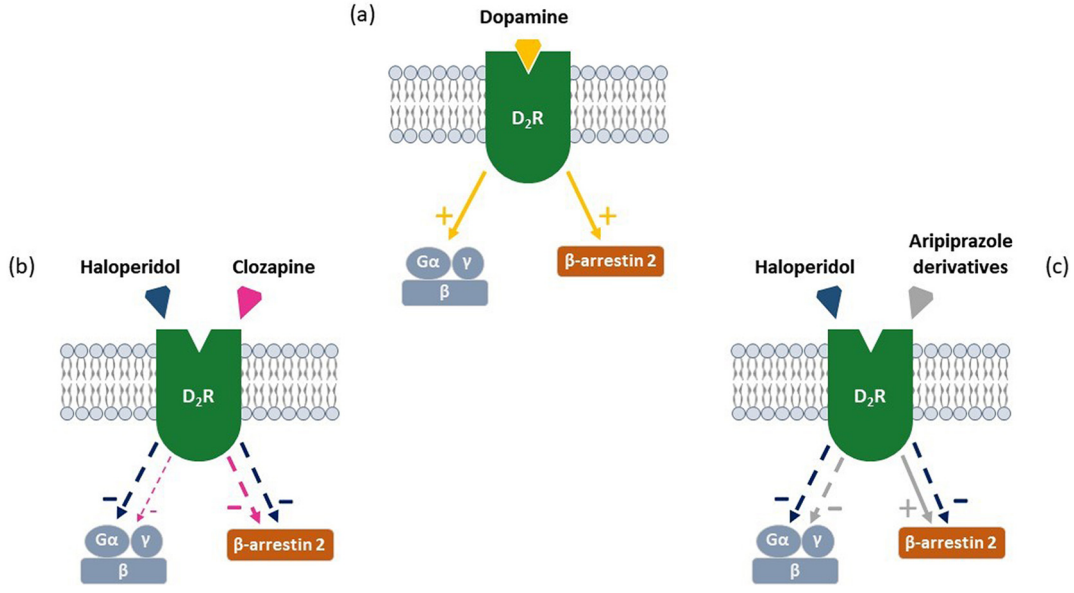

Fig. 3. Biased agonism at the D_2 receptor: potential role in the mechanism of AAPs. (a) The endogenous neurotransmitter dopamine is capable of activating both the G protein and the β-arrestin 2 pathways. (b) Haloperidol (TAP) has a strong affinity for the D_2 receptor, and it fully antagonizes both signaling pathways. On the contrary, clozapine in vitro seems to behave as a strong antagonist on the β-arrestin 2 pathway, while it acts as a weak inhibitor of the G protein pathway. (c) Recently, some derivatives of aripiprazole (UNC9975, UNC0006 and UNC9994) were synthesized and tested in vitro and in vivo, and they were found to behave as antagonists on the G protein pathway but to act as biased agonists on the β-arrestin 2 pathway.

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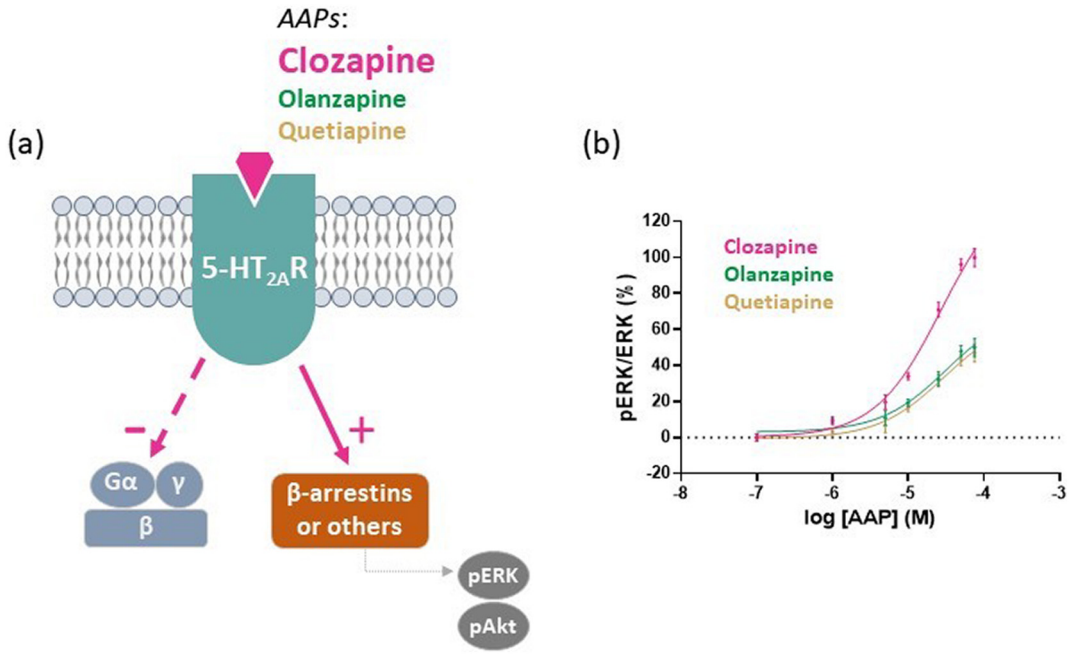

Fig. 4. Biased agonism at the 5-HT_{2A} receptor by clozapine and other AAPs. (a) Clozapine, and to a lesser extent olanzapine and quetiapine, act as biased agonists at the 5-HT_{2A} receptor, thereby activating intracellular pathways independent of G proteins, such as β-arrestin 2. This mechanism is responsible for activating ERK1/2 and Akt kinases, in vitro and in animal models of schizophrenia. Conversely, all three AAPs antagonize the 5-HT_{2A}-induced G protein activation. (b) Concentration-response curves of 5-HT_{2A}-mediated ERK 1/2 phosphorylation in the presence of clozapine, olanzapine and quetiapine (Aringhieri et al., 2017).

eventually have fewer side effects. The role of dimers or higher order oligomers in schizophrenia has been investigated, and the action of APs on these receptor complexes has been taken into consideration. Striatal sections of postmortem schizophrenic patients display variations in dimer expression compared to healthy controls. For instance, increases in D_2 receptor expression and homodimeric fraction were reported in postmortem schizophrenic patients (Seeman & Kapur, 2000; Wang et al., 2010). Conversely, the glutamatergic mGlu3 receptor dimers were reduced in the PFC (Corti et al., 2007). However, the data are still too preliminary to draw any conclusion.

Related to the mechanism of action of APs, the most analyzed heterodimeric complexes are the pairs, D_1-D_2, D_2-D_3, D_2-A_{2A} (adenosine), 5HT_{2A}-D_2 and 5HT_{2A}-mGlu2 (Moreno, Holloway, & González-Maeso, 2013), and there is evidence of activity of AAPs on these receptor complexes' expression and/or signaling. The adenosine receptor subtype A_{2A} is coupled to G_s and it allosterically modulates the D_2 receptor activity (Fuxe et al., 2005).

The D_1-D_2 heteromer is thought to couple to a different G protein, the G_q protein, and drive PLC-dependent calcium mobilization. The increased activity of dopamine in schizophrenia may increase D_1-D_2 heteromer formation and therefore G_q-PLC signaling through the concomitant activation of both receptors, as seen in in vitro and in vivo (striatum) studies. Interestingly, clozapine was able to dissociate the D_1-D_2 dimer, thereby reducing the overstimulation of PLC and intracellular calcium levels. The action of clozapine is effective at low concentrations due to its high affinity to the D_1-D_2 receptor complex (Dziedzicka-Wasylewska, Faron-Górecka, Górecki, & Kuśmider, 2008; Faron-Górecka, Górecki, Kuśmider, Wasylewski, & Dziedzicka-Wasylewska, 2008). Along with D_1-D_2, the D_1-D_3 and D_2-D_3 heteromers have also been taken into consideration. Previously, Scarselli et al. (2001) demonstrated in vitro a synergistic interaction between the D_2 and D_3 receptors forming a complex with high affinity for dopamine with unique functional properties. On the D_2-D_3 heteromers, aripiprazole and norclozapine, which are partial agonists on D_2 receptors, acted as potent antagonists that might contribute to their AP effect. The data suggest that these two compounds may have different pharmacological characteristics depending on the presence of heterodimeric complexes that may be different in dorsal versus ventral striatum (Maggio & Millan, 2010; Maggio, Scarselli, Capannolo, & Millan, 2015). Two studies have found interactions between D_1 and D_3 receptors forming a heteromeric complex, where the D_3 receptor agonists increase the affinity for D_1 receptor agonists and potentiate D_1 receptor agonist-mediated signaling through adenylyl cyclase (Fiorentini et al., 2008; Marcellino et al., 2008). Guitart et al. (2014) showed that allosteric interactions between these two receptors led to selective modulation of MAPK signaling and recruitment of β-arrestin 1. These data add further complexity to D_1 signaling, however the pharmacology of the D_1-D_3 heteromer in relation to APs is not yet known. Finally, there are several reports about D_2-D_4 heteromers and how they are able to modulate glutamate release (Borroto-Escuela et al., 2011; González, Rangel-Barajas, et al., 2012). A systematic study of the effects of APs on these heteromers has not been done.

In addition, interactions with non-dopaminergic receptors have also been reported and a possible role for A_{2A}-D_2, NMDA-D_2 and D_2-mGlu5 receptor heterodimers have been proposed (Borroto-Escuela et al., 2016). The A_{2A}-D_2 receptor heterodimer has been studied in relation with the pharmacology in PD and schizophrenia. The A_{2A} receptor agonists acted as APs in rat models through their antagonism on D_2 receptor-mediated $G_{i/o}$ signaling downstream the heteromer in the striatopallidal GABAergic neurons (Borroto-Escuela et al., 2016). This heterodimer most likely can interact with other receptors to form hetero-oligomeric complexes, such as the A_{2A}-D_2-mGlu5 complex. Some data indicate the existence of such oligomers on striatopallidal GABA neurons. Fuxe et al. (2008) proposed that concomitant treatment with A_{2A} and mGlu5 receptor agonists could be a new strategy for schizophrenia treatment via this complex. Moreover, glutamate activity is further complicated by the existence of D_2-NMDA as well as NMDA-mGlu5 receptor complexes. Fuxe et al. (2008) suggested a dynamic balance between mGlu5-NMDA and D_2-NMDA heterodimers, where the mGlu5-NMDA-D_2 complexes may transiently form as intermediates (Borroto-Escuela et al., 2016). The mGlu5 has also been shown to potentially form higher order complexes with A_{2A} and D_2 receptors in the rodent striatum, but their validation and relevance in psychotics is yet to be tested (Cabello et al., 2009).

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Given the role of both dopamine and serotonin in the mechanism of action of AAPs, a possible interaction between their receptors has also been considered. Notably, the presence of $5HT_{2A}$-D_2 heteromers was found in the ventral striatopallidal GABA pathway, PFC and pars reticulata of substantia nigra of rat brain (Łukasiewicz, Faron-Górecka, Kędracka-Krok, & Dziedzicka-Wasylewska, 2011). In vitro data indicated that concomitant stimulation of these two receptors in the heterodimeric complex enhanced PLC activation, while the D_2 receptor-mediated inhibition of adenylyl cyclase was diminished by co-stimulation of 5-HT_{2A} receptors through a trans-inhibition mechanism (Borroto-Escuela et al., 2010). In a cellular system expressing both 5-HT_{2A} and D_2 receptors, the high affinity site of the 5-HT_{2A} receptor for clozapine was no longer detectable due to its interactions with the D_2 receptor (Łukasiewicz et al., 2011).

In other in vitro studies, interactions between the 5-HT_{2A} and D_2 receptors were studied by comparing them with the known genetic variant $5HT_{2A}$(H452Y), and the effect of some APs was evaluated. The heteromeric $5HT_{2A}$(H452Y)-D_2 fraction was reduced compared to the wild-type counterpart $5HT_{2A}$-D_2, as evidenced by fluorescence resonance energy transfer measurements. In these experiments, clozapine, and not haloperidol, was able to restore the fraction of $5HT_{2A}$(H452Y)-D_2 heteromer at a level similar to the $5HT_{2A}$-D_2 receptor complex (Łukasiewicz et al., 2011). For the moment, the clinical consequences related to this are still not clear as only few studies have proposed that the $5HT_{2A}$(H452Y) polymorphism may be responsible for different clinical responses to AAP treatment (Wilffert, Zaal, & Brouwers, 2005).

Another receptor complex that seems to be involved in schizophrenia is the $5HT_{2A}$-mGlu2 heteromer of the somatosensory cortex in mice. In particular, as shown by studies in vitro and in vivo, the $5HT_{2A}$-mGlu2 complex enhances the activity of the 5-HT_{2A} component towards G_i, and less on G_q (Fig. 5a), and the activation of the mGlu2 component of this receptor complex arrests the hallucinogenic properties induced by 5-HT_{2A} receptor agonists, like lysergic acid diethylamide. Mechanistically, the mGlu2 monomer has an allosteric negative effect on 5-HT_{2A}-mediated $G\alpha_{q/11}$ activation, while enhancing its $G_{i/o}$ activity. Intriguingly, in the postmortem cortex of schizophrenic patients there is an increase of 5-HT_{2A} receptor expression and a decrease of mGlu2 receptors, which may be relevant to the pathogenesis of the disease. Also, chronic use of clozapine, and not haloperidol, in mice induced a downregulation of both 5-HT_{2A} and mGlu2 receptors in the somatosensory cortex (González-Maeso et al., 2008). Following these initial studies, Fribourg et al. (2011) demonstrated that the $5HT_{2A}$-mGlu2 heteromer is crucial to determine the coupling to $G_{i/o}$ or $G_{q/11}$, and different drugs may switch either to one or another signaling pathway. In schizophrenia, the mGlu2 downregulation and the 5-HT_{2A} upregulation may be associated with an increase of G_q coupling at the expense of G_i signaling and, in healthy animals, psychedelic drugs like 5-HT_{2A} agonists promote a similar switch (Fig. 5b). Conversely, in animal models of schizophrenia, AP medications like clozapine and risperidone invert the $5HT_{2A}$-mGlu2 heteromer activity in favor of G_i coupling, as it is in normal physiological conditions (Fig. 5c). In contrast, haloperidol was unable to revert such disruption (Fribourg et al., 2011). These results confirm the relevance of the $5HT_{2A}$-mGlu2 receptor complex in regulating the sensory functions in the somatosensory cortex, which may be disrupted in schizophrenia. Clozapine was able to restore the original function of this receptor complex with relevant consequences in animal models of schizophrenia. So far, this is the most compelling evidence of a possible role of heteromers in the mechanism of actions of AAPs. Among various strategies utilized to target pharmacologically receptor dimers, the use of bivalent ligands, targeting both monomers simultaneously, have received particular attention, and many bifunctional compounds have been synthesized that can label and discriminate the presence of dimers in vivo in animal tissues. This was demonstrated by McRobb, Crosby, Yuriev, Lane, and Capuano (2012) by using clozapine as a template to design a series of compounds where two molecules of clozapine were bound together with spacers of different length to label D_2 receptor dimers. However, the clinical use of these compounds is non-trivial as their pharmacokinetic properties are often unfavorable for in vivo administration. Intriguingly, on this topic, our group has discovered a compound, SB269,652, with dualsteric properties, which means that it acts

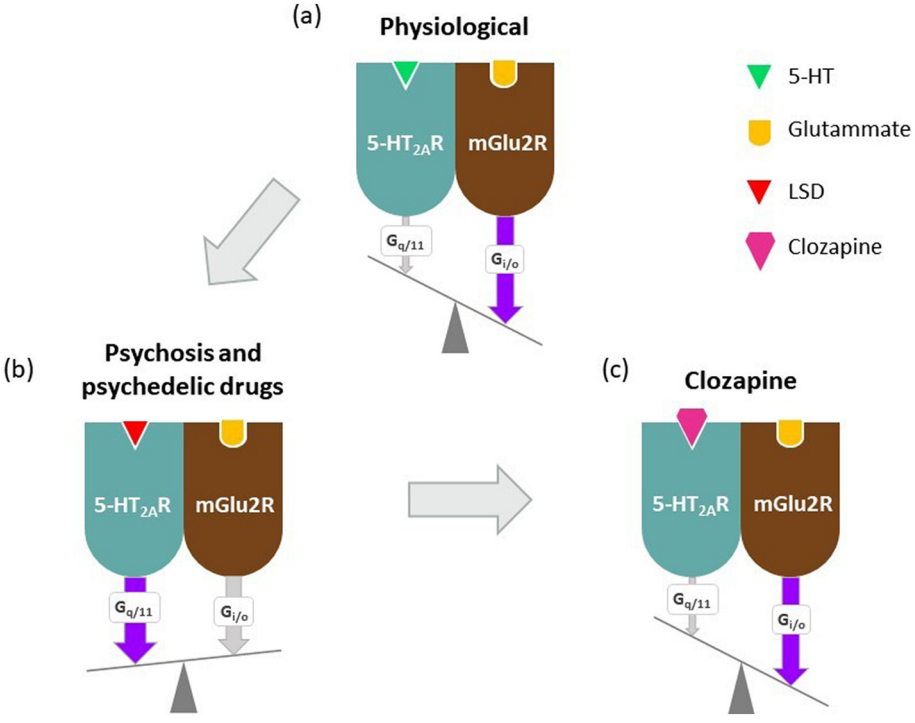

Fig. 5. Mechanism of action of AAPs at signaling of the $5HT_{2A}$-mGlu2 receptor complex. (a) In physiological conditions, the $5HT_{2A}$-mGlu2 receptor complex enhances the activity of 5-HT_{2A} towards G_i, and less on G_q. (b) In animal models, psychedelic drugs invert this balance by increasing G_q activity, and in schizophrenic patients mGlu2 receptor downregulation and 5-HT_{2A} receptor upregulation may lead to an increase of G_q coupling at the expense of G_i signaling. (c) Conversely, clozapine is able to restore the balance in favor of G_i coupling, as observed in physiological conditions.

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as an antagonist on the D_2 monomer, but as an allosteric negative modulator on the D_2 dimer (Rossi, Fasciani, Marampon, Maggio, & Scarselli, 2017; Silvano et al., 2010). Hence, this compound switches its antagonistic properties in favor of a mild negative allosterism in the presence of dimers, and this peculiar profile could offer some therapeutic advantages along with better tolerability in terms of side effects, such as parkinsonism and hyperprolactinemia (Carli et al., 2018). Finally, the use of so-called disrupting peptides has been an additional approach for targeting heteromers, where, instead of promoting or stabilizing the complex, peptides have been used to disrupt these complexes (Moreno et al., 2017; Viñals et al., 2015). They have been successfully deployed in vivo. However, as their in vivo stability is too short, they need to be optimized for their long-term delivery as therapeutics as suggested by Viñals et al. (2015).

Overall, these examples show how GPCR homo- and heteromerization provide new mechanisms to modulate GPCR signaling in physiological and pathophysiological conditions related to schizophrenia. However, even though these preliminary data look promising, there is a stringent need to find additional confirmation in vivo, and to discover new drugs that are able to interact exclusively with these receptor complexes.

4. Clinical differences among AAPs

In clinical practice, the question as to which AP should be preferred to ensure the highest probability of therapeutic success for treating schizophrenia or other psychoses is a complex and fascinating subject, as this mostly depends on the patient's condition and on the personal experience of the psychiatrist. However, many clinical studies have systematically compared AAPs with TAPs in terms of efficacy, quality of life, tolerability, drop out and side effects, and most of them demonstrated a better outcome with AAPs in several aspects (Leucht et al., 2009; Leucht et al., 2013; Leucht et al., 2017). Though, not all AAPs have achieved the same results, and among them, only clozapine, olanzapine, risperidone and amisulpride have systematically been shown to have an improved pharmacological profile in the treatment of positive and negative symptoms of schizophrenia compared to the prototypical TAP haloperidol, with clozapine being the most effective (Leucht et al., 2009). However, the clinical differences became less evident when haloperidol was used at lower doses, particularly in comparison with risperidone, although when confronted with the other three AAPs, in particular with clozapine, the differences still persisted, at least to a certain degree (Leucht, Wahlbeck, Hamann, & Kissling, 2003). This confirms the uniqueness of clozapine's clinical effect based on its ideal activity on dopamine and serotonin receptors, strongly in favor of the second, and also on other targets, i.e. muscarinic and noradrenergic receptors, glycine transporter and BDNF (Lieberman et al., 2005). Conversely, risperidone's activity is mostly based on a similar antagonism at dopamine and serotonin receptors, making it the least atypical in the family of AAPs. For these reasons, we propose a continuum spectrum of atypia that ranges from risperidone, the least atypical, to clozapine, the most atypical, while all other AAPs fall within these extremes of the spectrum (Fig. 6). On the other side, this characteristic makes risperidone a strong AP, very efficacious against psychotic symptoms of schizophrenia with pharmacological properties in certain aspects similar to that of haloperidol (Komossa et al., 2011). Similar considerations can be made for amisulpride, whose atypical characteristics at low doses become less evident at higher doses (Curran & Perry, 2002). Indeed, amisulpride's receptor profile is limited, mostly active on D_2/D_3 (medium D_2 k_{off}) and on 5-HT_7 receptors.

On the same topic, a very large meta-analysis, focused on a period of about 2 months, was carried out through direct and indirect comparison of the 15 most commonly used APs, including many AAPs and the two prototypic TAPs haloperidol and chlorpromazine. Regarding the overall activity, clozapine was significantly more effective than all other APs followed by amisulpride, olanzapine and risperidone, while quetiapine and aripiprazole together with new AAPs showed an overall efficacy similar to that of haloperidol and chlorpromazine (Leucht et al., 2013).

Despite considerable progresses in the pharmacological treatment of schizophrenia, about 1/3rd of patients are refractory to treatment, leading to increased morbidity and mortality. On treatment-resistant patients, clozapine is superior to all other AAPs, and since its discovery is still considered the 'gold standard' for treatment-refractory schizophrenia (Table 1) (Siskind, McCartney, Goldschlager, & Kisely, 2016). Clinical studies have confirmed that clozapine is the treatment of choice not only in treatment-refractory schizophrenia, but also for patients

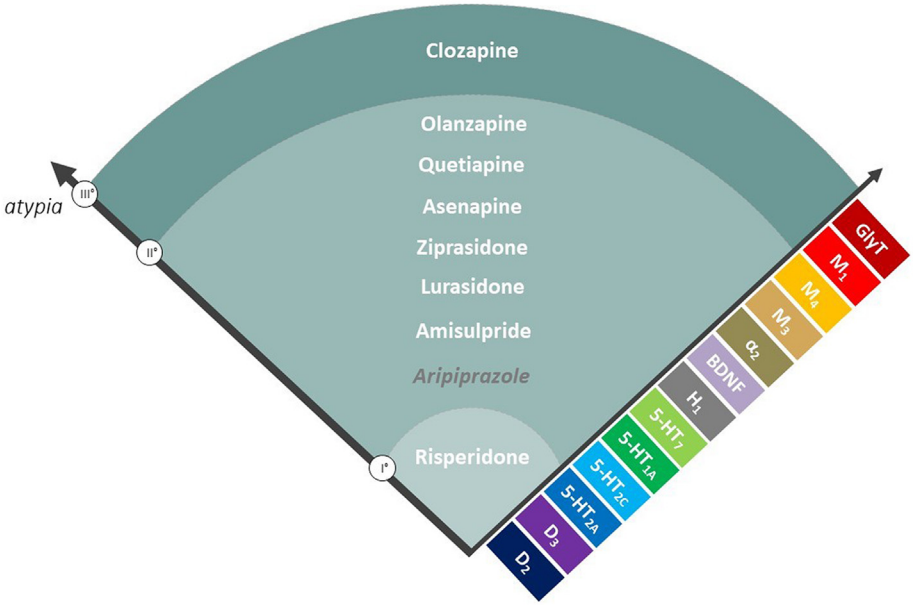

Fig. 6. Continuum spectrum of atypia: the three levels of atypicality. Based on the molecular profiles presented in Fig. 1, we propose to classify the AAPs in three categories, where risperidone is least atypical (Level I) and clozapine is most atypical (Level III), while all others fall within these two extremes of the spectrum (Level II). The molecular targets shown on the right add up, beginning with the D_2 and 5-HT_{2A} receptors that are common targets for all AAPs, extending to additional mechanisms such as M_1 positive allosterism and GlyT activity that seem specific to clozapine. Further targets, such as H_1 and α_2 receptors and BDNF, are relevant to both Level II and III of atypia. The clinical characteristics of each AAP are well explained according to their molecular profile on different targets.

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Table 1
Clozapine, the gold standard AAP: pros and cons.

Pros	Cons
• Efficacy in treatment-refractory schizophrenia • Efficacy on negative and cognitive symptoms (improved verbal fluency) • Efficacy in psychoses associated with PD • Efficacy in patients who develop TD • Only FDA-approved AAP to lower suicide risk and to exert some antidepressant properties • Diminished aggressive behaviors • No EPS • No TD • No increase in serum prolactin	• Agranulocytosis (0.7-1%) • Weight gain • Hyperglycemia, increase in triglycerides • Sialorrhea • Risk of epileptic seizure • Risk of myocarditis • Sedation

who display violent behaviors and/or are at high risk of suicide (Fakra & Azorin, 2012).

Apart from clinical differences among APs in terms of their efficacy, they differ in their side effects for which there is consensus in the scientific community. Regarding motor side effects, there is a continuum among AAPs starting from clozapine that practically never shows EPS and ending with risperidone that shows a notable rate of parkinsonism compared to others, especially at higher dose (Leucht et al., 2013). Similar to clozapine, quetiapine also never shows EPS, and for olanzapine this adverse event is quite rare. Another relevant motor side effect that concerns psychiatrists in the use of APs, particularly in the long term, is the occurrence of TD, a potentially irreversible movement disorder, the pathophysiology of which is not yet well understood (Lerner, Miodownik, & Lerner, 2015). The prevalence of TD in patients exposed to APs is about 20% after one year, with a cumulative increase of 5% per year during AP exposure (Stegmayer, Walther, & van Harten, 2018; Yassa & Jeste, 1992). The introduction of AAPs has been associated with a strong reduction of TD, however they still may cause TD, and this possibility should not be underestimated (Woods et al., 2010). The incidence of TD is about 2 to 10 times less for AAPs than for TAPs, depending on the study analyzed, and therefore there is a general consensus to prefer AAPs for long-term treatment. Among the AAPs, clozapine has the least propensity to induce TD, though it is yet to be determined whether the new AAPs, like ziprasidone, lurasidone and asenapine, are associated with a reduction of TD (Scarff & Casey, 2011). Strikingly, there is clinical evidence that clozapine very rarely causes TD, and moreover it may have beneficial effects on patients who develop this long-term motor complication. Hence, clozapine should be considered for patients who develop TD while receiving other APs (Bassitt & Louzã Neto, 1998).

Though in terms of motor side effects and hyperprolactinemia the AAPs are superior to TAPs, unfortunately the AAPs cause weight gain and other metabolic problems. For instance, olanzapine and clozapine treatments are associated with the greatest risk of weight gain, whereas quetiapine, risperidone and amisulpride show low-to-moderate levels of this undesired effect. Interestingly, the new AAPs such as ziprasidone, lurasidone and asenapine seem to have a low likelihood to cause these side effects. However, this advantage has to be balanced against their therapeutic efficacy, as these new drugs seem to be less effective in treating psychosis when compared to other AAPs (Leucht et al., 2017). As mentioned previously, the mechanisms of action associated with weight gain are complex and they involve many receptors like H_1, 5-HT_{2C} and other 5-HT receptors, and D_2 receptors. Interestingly, a polymorphism on the 5-HT_{2C} receptor was proposed to predict weight gain (Sicard et al., 2010; Zhang & Malhotra, 2013). Recent evidence supports nutritional interventions and psychoeducational programs for preventing AP-induced weight gain (Curtis et al., 2016). This approach was associated with lesser weight gain in participants treated with olanzapine (Jacobowitz, Derbabian, & Saunders, 2014). Different programs that included nutrition, physical activity and psychoeducation, have been shown to be useful in reducing weight in a clinical population taking APs (Magni et al., 2017).

Regarding metabolic problems, clozapine, and especially olanzapine, may be associated with hyperglycemia and dyslipidemia, hence they should be avoided in diabetic and/or obese patients (Table 1). These side effects are in part a consequence of weight gain and in part are due to mechanisms that involve both peripheral and central molecular targets. The blockade of hypothalamic 5-HT_{2C} and H_1 receptors results in increased appetite and weight gain, while M_3 receptor antagonism inhibits M_3 receptor-induced insulin secretion from the pancreatic beta cells, and therefore leads to hyperglycemia (Ballon et al., 2018; Liu et al., 2017).

In relation to side effects, it is also relevant to mention that AAPs, especially sertindole, might induce electrocardiogram alterations, like QTc prolongation, and for this reason patients should be carefully monitored (Beach, Celano, Noseworthy, Januzzi, & Huffman, 2013).

Dealing with other difficult situations present in schizophrenia, the negative symptoms (apathy, anhedonia, asociality) and cognitive impairment are relevant features that might profoundly affect clinical recovery and social rehabilitation. On negative symptoms, the data are consistently in favor of AAPs compared to TAPs, and this has been a turning point since the introduction of clozapine. There are mainly two reasons to explain these differences. First, TAPs worsen negative symptoms of schizophrenia because of their strong antagonism at D_2 receptors that has a negative impact on dopaminergic activity in the PFC. Second, AAPs increase dopamine, noradrenaline and ACh efflux in the PFC, which has a positive clinical outcome, although the deleterious effect of TAPs on negative symptoms is less detrimental at lower dosages. Differences within the AAP family are small, however a continuum of efficacy on negative symptoms, starting with risperidone (the least) and ending with clozapine and olanzapine (the most) has been found in different studies (Alvarez, Ciudad, Olivares, Bousoño, & Gómez, 2006). The suppression of negative symptoms may be influenced in part by reduction of positive symptoms, and this further complicates any clinical investigation (Czobor & Volavka, 1996).

Regarding the cognitive deficits associated with schizophrenia, AAPs may produce a mild remediation with differential effects on specific cognitive domains. Clozapine significantly improves verbal fluency more than any other AAP (Woodward, Purdon, Meltzer, & Zald, 2005), and quetiapine and olanzapine seem more effective in attention and processing speed (Désaméricq et al., 2014), while risperidone shows the least beneficial effects on these cognitive domains, which may be due to its high affinity for D_2 receptors (Nielsen et al., 2015). Conversely, the cholinomimetic properties of clozapine, through its metabolite norclozapine acting particularly on the M_1 receptor, may contribute to its favorable profile in cognition (Olianas, Maullu, & Onali, 1999; Zorn, Jones, Ward, & Liston, 1994). Interestingly, besides the anti-muscarinic activity of olanzapine, and also clozapine in part, these two AAPs do not appear to have a negative impact on patient's cognitive functions, at least at low-medium doses (Kennedy et al., 2001; Street et al., 2000). Alternatively, the possible cognitive enhancement induced by clozapine, olanzapine and quetiapine may be related to increased release of dopamine and other neurotransmitters in the PFC and hippocampus (Ichikawa, Li, Dai, & Meltzer, 2002; Shirazi-Southall, Rodriguez, & Nomikos, 2002). There is a number of clinical data indicating a modest effectiveness of AAPs in cognitive improvement (Davidson et al., 2009; Keefe et al., 2007; Nielsen et al., 2015; Vreeker, van Bergen, & Kahn, 2015).

Another aspect that needs to be addressed is the use of AAPs in psychoses associated with PD which may be caused either by the progression of this disease or by the use of L-Dopa or dopamine agonists (Zahodne & Fernandez, 2008). A number of studies have been conducted investigating the role of AAPs, like clozapine, quetiapine, olanzapine and risperidone, for this adverse effect, and among them, only clozapine, at low dose, demonstrated superiority over placebo in reducing the psychotic symptoms (Parkinson Study Group, 1999). In few open-label studies, patients treated with quetiapine experienced partial resolution of psychosis, but these data have not been confirmed

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in double-blind trials. Furthermore, the use of quetiapine was associated with a high prevalence of dropouts due to its adverse effects (Jethwa & Onalaja, 2015). Olanzapine and risperidone were unable to significantly improve psychotic symptoms, and in some cases they even exacerbated motor complications (Jethwa & Onalaja, 2015). On this topic, pimavanserin has recently been approved for psychoses associated with PD. Pimavanserin is a preferential 5-HT_{2A} receptor antagonist with some residual activity on 5-HT_{2C} receptors as well, but it is devoid of activities on almost all other receptors targeted by AAPs (Cummings et al., 2014; Sarva & Henchcliffe, 2016). These results support the high relevance of the 5-HT_{2A} receptor and, secondly, the 5-HT_{2C} receptor to attenuate psychoses in PD, a mechanism that may be shared, at least in part, by clozapine. However, the mechanism of action of clozapine on this aspect is probably more complex. Unfortunately, these favorable characteristics of clozapine have to be well balanced with its side effects, like agranulocytosis, weight gain and metabolic problems, all advocating a careful monitoring of the patients by clinicians.

In conclusion, clinical evidence has found important differences among AAPs, with clozapine being the best choice in different medical conditions, such as treatment-refractory schizophrenia, in psychoses associated with PD and in patients who develop TD (Table 1). Besides clozapine, AAPs like olanzapine, amisulpride and risperidone have also shown superiority compared to other APs, even if risperidone and amisulpride often present motor side effects and hyperprolactinemia. These diversities are quite well explained by their profiles on different molecular targets. The weight gain and metabolic problems, associated especially with clozapine and olanzapine, urge psychiatrists for a tailored therapy designed as per patient's condition.

5. Therapeutic drug monitoring (TDM) of AAPs: towards a personalized therapy

From prescribed dose to clinical drug response, multiple factors of a pharmacokinetic and pharmacodynamic nature are determinant for the therapeutic success of AAPs. Since the conception of personalized pharmacotherapy, strong efforts have been made to understand all interindividual variables that influence the therapeutic response, and to tailor the required dosage for individual patients. This is particularly relevant for psychiatric disorders where more than 1/3rd of the patients do not receive any benefit from the pharmacological treatment, and where 20-60% of the patients, in the long-term, suspend drug usage either due to side effects or for non-adherence (poor compliance).

Considering the high variability in drug metabolism among patients, TDM is a rational approach for optimizing and personalizing pharmacotherapy, where the drug plasma concentration (Cp) can be a relevant parameter for drug efficacy and tolerability. Some AAPs have shown a good correlation between their Cp and the highest probability of response with minimized risk of adverse drug reactions. Indeed, TDM of APs is particularly useful for identifying a non-response at therapeutic doses, uncertain drug adherence, pharmacokinetic drug-drug interactions and reduced side effects. In addition, for some particular categories of patients like children, adolescents, pregnant women, elderly individuals and persons with intellectual disabilities, TDM seems particularly useful (Hiemke et al., 2018).

For AAPs, the prediction of Cp after drug administration is difficult, and many interindividual factors affect this parameter (Grundmann, Kacirova, & Urinovska, 2014; Kornhuber, Wiltfang, Riederer, & Bleich, 2006; Mauri et al., 2001; Mauri et al., 2007). Many studies related to the variability between AAP dose and Cp have been done with clozapine, which nowadays is frequently monitored. The Cp of clozapine cannot be predicted due to large interindividual variability factors, such as sex, weight, smoking and concomitant use of other medications that influence CYP450 activity (e.g. CYP1A2) (Rostami-Hodjegan et al., 2004). In particular, with a fixed dose of clozapine of 400mg/day, Potkin et al. (1994) found a very large Cp variability, ranging from 40 to 1911 ng/ml. Ageing was also shown to increase the Cp of clozapine as its active metabolite norclozapine increases up to 72% in older patients (Castberg, Westin, Skogvoll, & Spigset, 2017). Sex related differences in Cp was reported to be higher in females (Castberg et al., 2017; Mauri et al., 2004). On the contrary, smoking lowers the Cp of clozapine by inducing CYP1A2 (Lopez & Kane, 2013). Fluvoxamine was shown to increase the Cp of clozapine up to 10 times, and this is related to its inhibitory activity on CYP1A2. On the other hand, co-administration with carbamazepine, a CYP3A4 and CYP1A2 inducing drug, resulted in a substantial decrease in the Cp of clozapine (Jerling, Lindström, Bondesson, & Bertilsson, 1994). Similar interactions were found with other AAPs like olanzapine and risperidone when they were co-administered either with SSRIs (e.g. fluoxetine and paroxetine), which are mostly CYP2D6 and CYP2C19 inhibiting drugs, or with carbamazepine (Spina & de Leon, 2007). Genetic variants regarding the CYP450 family could also explain some Cp variability and efficacy among AAPs (Pouget, Shams, Tiwari, & Müller, 2014). For example, several studies have shown that CYP2D6 polymorphisms may influence the efficacy of risperidone, however these data are controversial (Almoguera et al., 2013; Kakihara et al., 2005).

Neuroimaging studies have demonstrated that EPS may occur when more than 80% of D_2 receptors in the striatum are blocked. Importantly, a correlation was found between the D_2 receptor occupancy and the Cp of the APs, whereas such a relationship with dosage was less clear. Indeed, the Cp is a good predictor for its cerebral concentration (Hiemke et al., 2011), especially for lipophilic drugs where the blood-brain barrier efflux transporters are poorly involved. A recent finding confirmed a good correlation between Cp and D_2 receptor occupancy of AAPs in striatal areas (Grundmann et al., 2014).

Other studies have found that the relationship between Cp and D_2 receptor occupancy is nicely fit by a hyperbolic saturation curve (one site model), where risperidone and olanzapine, at higher concentration, may exceed 80% of receptor occupancy, while clozapine or quetiapine never reach this level (Lako, van den Heuvel, Knegtering, Bruggeman, & Taxis, 2013; Uchida et al., 2011). These curves show a good correlation between predicted and observed receptor occupancy in relation to the drug Cp. The prediction of D_2 receptor occupancy in relation to Cp is particularly valid for olanzapine, less for risperidone and not significant for clozapine. For risperidone, blood-brain barrier efflux transporters such as P-glycoprotein (P-gp) may be responsible for lowering its concentration in the brain, thus reducing the above mentioned correlation, while in the case of clozapine, the lack of this correlation may be due to its lower affinity for the D_2 receptor. Interestingly, P-gp pharmacogenetics contribute to the efflux of APs from the CSF, and three different polymorphisms have been associated with the variation in AP efficacy (Pouget et al., 2014), particularly for risperidone. The expression of P-gp is controlled by many factors besides the genetic background, such as pathophysiological conditions, hormones and diet (Miller, 2015).

The effect of APs on D_2 receptor occupancy was also studied in extrastriatal regions. In the case of clozapine, Gründer et al. (2006) showed a larger occupancy for cortical receptors than striatal ones at clinically significant Cp. However, the data are controversial and Agid et al. (2007), for olanzapine and risperidone, found a correlation between clinical outcomes and receptor occupancy only in striatal, but not extrastriatal regions. Recently, some in vivo studies have analyzed the possible relationship between Cp and receptor occupancy for other targets such as the 5-HT_{2A} receptor in the cortex and GlyT1 transporters, however the information is still too preliminary (Alberati et al., 2012; Mamo et al., 2004).

Regarding drug efficacy, several studies have found a good correlation between AP response and its Cp, especially for clozapine and olanzapine. In fact, TDM of these two drugs is strongly recommended as indicated by the *Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie* consensus guidelines (Level I recommendation) (Hiemke et al., 2018).

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Perry, Miller, Arndt, and Cadoret (1991) for the first time showed in treatment-resistant schizophrenic patients that a Cp of clozapine greater than 350 ng/ml resulted in a 64% clinical response, while below this level the response was only 22%. Other studies have also confirmed a cut off for clozapine efficacy at 350 ng/ml (Kronig et al., 1995; Perry, 2000) or 420 ng/ml (Mauri et al., 2007; Potkin et al., 1994; Spina et al., 2000). However, this evidence has not always been confirmed due to the complexity and variability of the analysis (Dettling et al., 2000).

In addition, a correlation was found between Cp of clozapine and increased risk of epilectic seizures, and hence the proposed therapeutic range is currently 350–600 ng/ml, with an upper alert limit of 1000 ng/ml (Hiemke et al., 2018; Mauri et al., 2007). A concentration above 1000 ng/ml increases the risk of delirium, confusion and seizures (Grundmann et al., 2014). In addition, a fluctuation of clozapine Cp is predictive for relapses and re-hospitalization in schizophrenic patients, where TDM may reduce such risks and show important cost-effective advantages (Hiemke et al., 2018). There is also some evidence for a distinct relationship between Cp and clinical efficacy of olanzapine and risperidone.

In conclusion, several data have pointed out the utility of TDM for clinical use of AAPs as stated by the *Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie* consensus guidelines, particularly for clozapine and olanzapine (Hiemke et al., 2018). In addition, the relatively narrow therapeutic range of clozapine suggests that in most individuals, besides any intervariabilities, there is a clinical response above a Cp of 350 ng/ml. Does this mean that clozapine, among the many receptors targeted, has just one or some few specific mechanisms? Or does it mean that the many targeted receptors converge in something specific? Intriguingly, lithium also has a narrow therapeutic window with a threshold at 0.5 mM. These questions demand further investigation into the mechanisms of action of AAPs in relationship with their pharmacokinetic and pharmacodynamic properties.

6. Conclusions

In this review, we have highlighted the pharmacological differences among the AAPs with the scope to find a link between the molecular targets of AAPs and their clinical characteristics. Many have questioned the classification of APs into the two classes of TAPs and AAPs, underlining that each AP shows unique characteristics. In fact, in clinical practice, many psychiatrists are inclined toward a tailored therapy according to the patient's characteristics and risks of side effects. As a consequence, treatment is mainly decided by trying to avoid the risk of motor side effects, weight gain and other metabolic issues associated with APs.

However, despite the inherent variety among all APs, we still believe that the classification of AAPs is an important reference for research and clinical use alike. The concept of atypia is still intact in its essence and refers to a category of APs (AAPs) which demonstrate reduced motor problems, reduced hyperprolactinemia, and reduced worsening of apathy and anhedonia along with a possible improvement of negative and cognitive symptoms of schizophrenia. Other therapeutic advantages relate to efficacy in treatment-refractory schizophrenia, psychoses associated with PD and TD. In all these conditions, clozapine may be considered as the gold standard of AAPs.

In addition, in order to reconcile the concept of atypicality and the diversity of each AAP, we propose a continuum spectrum of atypia that ranges from risperidone, the least atypical, to clozapine, the most atypical, while all the other AAPs fall within the extremes of this spectrum (Fig. 6). It is worth mentioning that risperidone and amisulpride can lose their atypicality at higher doses.

Importantly, the clinical characteristics of each AAP could be predicted by their molecular profile on different targets. For instance, the ratio of 5-HT_{2A}/D_2 and 5-HT_{2C}/D_2 receptor affinity together with a rapid k_{off} from the D_2 receptor are two important factors that distinguish AAPs in terms of efficacy and side effects. However, these two mechanisms are not mutually exclusive, considering the relevance of 5-$HT_{2A/2C}$ receptors for regulating dopamine release in the synaptic cleft. Intriguingly, some AAPs were shown to have biased signaling activities at D_2 and 5-HT_{2A} receptors, and therefore are able to preferentially activate a specific receptor-mediated intracellular signaling pathway. For instance, in some experimental models, clozapine has been shown to act as a biased agonist at the 5-HT_{2A} receptor and to activate ERK and Akt, although the clinical consequences of these effects are yet to be determined.

Besides D_2 and 5-$HT_{2A/2C}$ receptors, other molecular targets are relevant to further characterize the AAPs, and among them, 5-HT_1 partial agonism, D_3 antagonism, H_1 antagonism, α_2 antagonism, muscarinic antagonism (moderately), M_1 positive allosterism, BDNF production and GlyT blocking have received particular attention. Clozapine has a unique profile on these molecular targets and this might explain its broad clinical activity. Moreover, this raises many questions: are all these molecular targets equally relevant to explain atypia or are some more important than others? Do the many targeted receptors converge in some specific cellular mechanisms? Are there still some undiscovered molecular targets? These outstanding questions demand further investigations, and the answers will allow a better understanding of the mechanism of atypia and to find new ways to develop better drugs.

Conflict of interest

The authors declare that there is no conflict of interest related to this publication.

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