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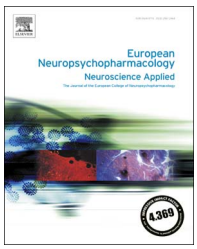

Clozapine as the most efficacious antipsychotic for activating ERK 1/2 kinases: Role of 5-HT_{2A} receptor agonism

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Abstract
Antipsychotics (APDs) are divided into first-generation antipsychotics (FGAs) and second-generation antipsychotics (SGAs) based on the concept that SGAs have reduced motor side effects. With this premise, this study examined in HeLa and other cell lines the effects of different APDs on the activation of ERK1/2 (Extracellular signal-regulated kinases) and AKT (Protein Kinase B) kinases, which may be affected in schizophrenia and bipolar disorder. Among the SGAs, Clozapine clearly resulted as the most effective drug inducing ERK1/2 phosphorylation with potency in the low micromolar range. Quetiapine and Olanzapine showed a maximal response of about 50% compared to Clozapine, while FGAs such as Haloperidol and Sulpiride did not have any relevant effect. Among FGAs, Chlorpromazine was able to partially activate ERK1/2 at 30% compared to Clozapine. Referring to AKT activation, Clozapine, Quetiapine and Olanzapine demonstrated a similar efficacy, while FGAs, besides Chlorpromazine, were incapable to obtain any particular biological response. In relation to ERK1/2 activation, we found that 5-HT_{2A} serotonin receptor antagonists Ketanserin and M100907, both partially reduced Clozapine effect. In addition, we also observed an increase of potency of Clozapine effect in HeLa transfected cells with recombinant 5-HT_{2A} receptor and in rat glioma C6 cells that express a higher amount of this receptor. This indicates that ERK1/2 stimulation induced by Clozapine could, to some extent, be

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mediated by 5-HT_{2A} receptor, through a novel mechanism that is called "biased agonism", even though other cellular targets are involved. This evidence may be relevant to explain the superiority of Clozapine among the APDs.

1. Introduction

Antipsychotics (APDs) are widely prescribed drugs for schizophrenia and bipolar disorder, brain diseases that are characterized by psychotics features (Miyamoto et al., 2012). Generally, these drugs are divided into first-generation antipsychotics (FGAs), called typical antipsychotics, and second-generation antipsychotics (SGAs), called atypical, based on the concept that SGAs have reduced side effects such as Parkinsonism and tardive dyskinesia (Meltzer, 2013). Moreover, SGAs seem to have additional therapeutic properties such as cognitive enhancement, improvement of negative symptoms, and most importantly, prevention of progressive clinical deterioration. Recent findings suggest that SGAs might possibly slow down the loss of gray matter in schizophrenic patients at early stages as a further demonstration of their unique pharmacological profile and curative benefits (Lieberman et al., 2005; Van Haren et al., 2007).

In fact, in animal models some SGAs have shown to induce neurogenesis, synaptogenesis and increase in neurotrophins (Lieberman et al., 2008). However, to find a direct relationship between pharmacodynamics characteristics and therapeutic properties for APDs, particularly among SGAs, is complicated as these drugs have very complex receptor profiles. Many hypotheses have been formulated on this topic (Miyamoto et al., 2005). In general, the mechanism of action of typical antipsychotics is to block dopamine D_2 receptor, while for atypical other explanations have been proposed besides D_2 receptor. Indeed, for atypical antipsychotics, many works have pointed out the importance of other G protein-coupled receptors (GPCRs) such as 5-HT_{2A} and 5-HT_{1A} serotonin receptors and also muscarinic, adrenergic, glutamatergic and histamine receptors (Meltzer and Massey, 2011). Furthermore, although acute events, such as psychoses, are probably controlled by short-term effects of APDs mostly mediated by their receptor affinities, it is evident that these drugs have more complex effects, particularly in the long-term time scale, involving intracellular mechanisms that may regulate neuronal functionality and plasticity (Molteni et al., 2009; Fumagalli et al., 2009).

Among these additional intracellular mechanisms responsible for the APDs pharmacological action, kinases such as ERK1/2 (extracellular signal-regulated kinases) and AKT (protein kinase B) have received particular attention (Molteni et al., 2009; Freyberg et al., 2010). ERK1/2 pathway represents a fundamental crossroad of multiple signaling cascades involved in regulating many cellular activities induced by different receptors, either receptor tyrosine kinases (RTKs) or GPCRs (Rubinfeld and Seger, 2005). Cussac et al. (2002) showed the activation of ERK in CHO cells after treatment with Clozapine. Same results were described in the dorsal striatum of rat after the infusion of Haloperidol, while Clozapine reduced the activation of ERK1/2 (Pozzi et al., 2003). Regarding the Central Nervous System (CNS), ERK1/2 activity is relevant for synaptogenesis, neurogenesis, connectivity and neural plasticity, processes that are implicated in schizophrenia (Samuels et al., 2008, 2009). Similar to ERK1/2, AKT is one of the survival kinases with multiple biological functions in the brain and the whole body. For the CNS, AKT is important in neurodevelopment, synaptic plasticity, protein synthesis and neurotransmission (Shioda et al., 2009; Arguello and Gogos, 2008). Interestingly, AKT activation induces the phosphorylation of other kinases, such as GSK-3β, that are relevant in psychiatric disorders (Beaulieu et al., 2009). GSK-3β has recently been proposed as a contributing factor in the etiology of schizophrenia and bipolar disorder (Emamian, 2012). Lu et al. showed for the first time a role for these kinases, activated by some SGA, in neuronal survival and neurite outgrowth (Lu et al., 2004, Lu and Dwyer, 2005).

If the debate on differences between FGAs and SGAs is still an open topic, there is another ongoing discussion within this debate that tries to understand the superiority of Clozapine among the SGAs (Meltzer, 2012). Clozapine is, in fact, the most efficacious drug in treating positive and negative symptoms in schizophrenia, particularly in patients resistant to other antipsychotics, and it is considered as the "gold standard" for treating schizophrenia (Leucht et al., 2013). Unfortunately, its benefits are outweighed by relevant side effects such as the risk of severe hematological effects and metabolic syndrome (Capannolo et al., 2015). What makes Clozapine so unique compared to all other APDs is still unclear, and is probably related to its multifactorial properties on receptors and other targets. Recently, a new concept regarding GPCR function called "biased agonism" has gained attention wherein a drug can behave either as agonist or as antagonist on the same receptor depending on the specific pathway that is taken into consideration (Kenakin, 2011; Reiter et al., 2012). For a fact, Clozapine is an antagonist on 5-HT_{2A} serotonin receptor in relation to the G protein activation but it is an agonist on the same receptor if AKT activation is measured, as previously demonstrated by Schmid et al. (2014). With these premises, our study has compared in different cell lines, in particular in HeLa cells, the activity of FGAs and SGAs on ERK1/2 and AKT kinases. In addition, to understand their mechanism of action, we also investigated the role of 5-HT_{2A} serotonin receptor and other GPCRs. Our experiments have demonstrated that Clozapine has a unique profile, particularly in relation to ERK1/2 activation.

2. Experimental procedures

2.1. Reagents: chemicals and antibodies

Clozapine, Quetiapine, Olanzapine, Risperidone, Aripiprazole, Chlorpromazine, Haloperidol, Sulpiride, Serotonin, α-methyl-serotonin, PMA (phorbol 12-myristate 13-acetate), hEGF, FR180204, PD184352, GSK690693, GSK2334470, Ketanserin, M100907, Marimastat,

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