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PERSPECTIVE

Messing Up with Traffic: Different Effects of Antipsychotic Agents on Glutamate Receptor Complexes in Vivo

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ABSTRACT

Antipsychotic agents are major drugs for human neuropsychiatric conditions including schizophrenia, mood disorders, Tourette syndrome, and Alzheimer's disease. These drugs are divided in two groups—first-generation/typical and second-generation/atypical—on the basis of their propensity to induce extrapyramidal motor side effects. Furthermore, second-generation antipsychotics have been reported to be superior in addressing cognitive deficits in schizophrenia. Understanding differences between the mechanism of action of first- and second-generation antipsychotic agents thus represents an interesting opportunity for the development of new compounds having better therapeutic action and less side effects. In this issue of *Molecular Pharmacology*, Fumagalli et al. (p. 1484) report that long-term treatment with the first-generation drug haloperidol interferes with the trafficking of both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and *N*-methyl-D-aspartate glutamate receptor complexes and associated molecules post-synaptic densities 95 and Ca^{2+}calmodulin-dependent protein kinase in the rat frontal cortex. In contrast, the second-generation drug olanzapine did not affect glutamate receptor trafficking. The action of haloperidol on glutamate receptor trafficking in specific brain regions may contribute to the low efficacy of this drug on cognitive deficits and to the development of side effects. Overall, antipsychotics have been shown to act upon multiple signaling mechanisms (e.g., cAMP-protein kinase A, βArrestin 2-Akt-GSK-3, and phospholipase C-inositol-protein kinase C pathways), mostly by blocking D2-class dopamine receptors (first generation) or D2-class dopamine and 5-HT_2 serotonin receptors (second generation). Identification of specific pathways by which haloperidol affects glutamate receptor trafficking may thus represent an important next step toward the development of better antipsychotic drugs.

Since the introduction of chlorpromazine in the 1950s, antipsychotic agents have become the principal therapeutic intervention for schizophrenia. Moreover, these drugs are sometimes used for the management of other neuropsychiatric conditions, such as mood disorders, Tourette syndrome, and Alzheimer disease. Antipsychotic agents are divided in two broad categories under the basis of their effects and side effects (Meltzer, 1991; Kapur and Remington, 2001). First-generation (FGA) or typical antipsychotic agents such as chlorpromazine or haloperidol are associated with the development of extrapyramidal side effects. In contrast, second-generation (SGA) or atypical antipsychotics (e.g., clozapine, olanzapine) have a lower incidence of extrapyramidal side effects while still exerting therapeutic action. Some studies have also indicated that SGAs may be superior to FGA in improving cognitive deficits in persons with schizophrenia (Keefe et al., 2006; Di Pietro and Seamans, 2007). However, SGAs are not without problems as their administration can lead to metabolic complications such as diabetes and obesity (Haupt and Kane, 2007). Clozapine can also induce agranulocytosis (Idänpään-Heikkilä et al., 1975), a potentially fatal condition, thus requiring close supervision of patients' blood granulocyte levels. Furthermore, a recent large scale Clinical Antipsychotic Trials for Intervention Effectiveness (CATIE) has challenged the overall clinical superiority of SGAs over FGAs in clinical practice (Lieberman et al., 2005).

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ABBREVIATIONS: FGA, first-generation antipsychotic; SGA, second-generation antipsychotic; DA, dopamine; 5-HT, serotonin; GSK-3, glycogen synthase kinase 3; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-D-aspartate; PSD, postsynaptic density; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase; PP2A, protein phosphatase 2A.

In this context, understanding the molecular mechanisms by which SGAs and FGAs exert their therapeutic action and induce specific side effects is of a tremendous importance for the development of much needed new compounds with antipsychotic actions. At the receptor levels, FGAs are potent antagonists of D2-class dopamine (DA) receptors (Creese et al., 1976) while SGAs are weaker D2-class receptor antagonists while also blocking 5-HT_2 serotonin (5-HT) receptors (Meltzer, 1991; Roth et al., 2004; Sheng and Hoogenraad, 2007). Recent evidence has shown that some signaling molecules, notably the serine/threonine kinase glycogen synthase kinase 3 (GSK-3), can be regulated in a similar fashion by both SGAs and FGAs (Beaulieu et al., 2007). However, the link between these observations and the pharmacological actions of antipsychotics has not been clearly elucidated.

Ionotropic glutamate receptor complexes AMPA and NMDA are potentially important indirect targets for antipsychotics. These receptors are heterotetrameric ion channels whose expression and trafficking are tightly regulated by complex networks of scaffolding proteins and signaling molecules (Sheng and Hoogenraad, 2007). Noncompetitive NMDA/glutamate receptor antagonists, such as ketamine and phencyclidine, induce psychotic-like responses in human as well as in primate and rodent animal models (Sharp et al., 2001). Furthermore, mice expressing only 5% of the normal level of the NMDA receptor subunit NR1 (NR1KD mice) also display "schizophrenia-like" behaviors, such as enhanced stereotypy and locomotion as well as deficits in social and reproductive behaviors (Mohn et al., 1999). Importantly, these behavioral abnormalities can be ameliorated by antipsychotics in both NR1KD mice and in rodents treated with noncompetitive NMDA/glutamate receptor antagonists. This suggests that antipsychotics may indirectly affect the regulation of ionotropic glutamate receptor complexes by blocking DA and/or 5-HT receptor functions.

Few investigations have focused on the impact of antipsychotics on ionotropic glutamate receptor complexes trafficking in the brain. In an article published in this issue of *Molecular Pharmacology*, Fumagalli et al. (2008) used subcellular fractioning of brain tissue to examine the expression and trafficking of glutamate receptor complex proteins to post-synaptic densities (PSD) in response to chronic treatment with antipsychotics (Fumagalli et al., 2008). The authors administered repeated treatment of two antipsychotics, haloperidol and olanzapine, in adult rats. They found that the SGA olanzapine does not affect the expression or trafficking of NMDA receptor complexes in the frontal cortex and hippocampus. In contrast, long-term administration of the FGA haloperidol leads to reduced trafficking of NMDA receptor subunits NR1 and NR2A as well as AMPA receptor subunit GluR1 to PSDs in the rat frontal cortex. These finding expend a recent report by this same group of a similar reductions in NMDA receptor synaptic expression in the striatum in response to long-term haloperidol treatment (Gardoni et al., 2008).

The authors also examined the impact of long-term haloperidol treatment on the expression and trafficking of proteins associated to ionotropic glutamate receptor complexes. The Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) is a multimeric serine/threonine kinase involved in the regulation of synaptic plasticity and glutamate receptor trafficking (Hudmon and Schulman, 2002). Furthermore, it has been suggested that CaMKII may also act as a protein scaffold for the recruitment of other proteins to PSDs (Bayer et al., 2006). One important step in the regulation of CaMKII is its autophosphorylation on threonine 286 (thr286) that increases its affinity for calmodulin, thus resulting in an augmentation of kinase activity. Fumigalli et al. (2008) report that long-term haloperidol treatment interferes with the trafficking of phospho-thr286-CaMKII to PSD. Furthermore, haloperidol reduces the interaction of CaMKII with the NMDA subunit NR2B.

Scaffolding postsynaptic protein of the MAGUKs family (PSD95, SAP 102, chapsyn-110/PSD-93, and SAP97) (Garner and Kindler, 1996) interacts with NMDA receptor complexes

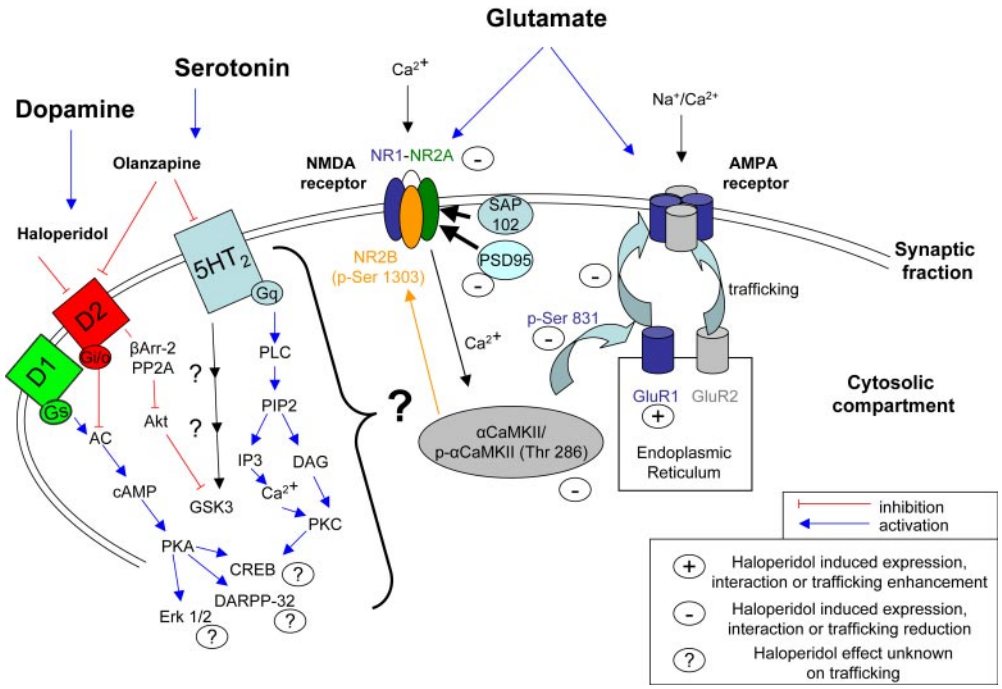

Fig. 1. Model of the haloperidol and olanzapine effects on ionotropic glutamate receptors trafficking. Signaling pathways directly affected by D2 and 5-HT2 receptors are also indicated.

(Sheng and Hoogenraad, 2007). It is noteworthy that reduced expression of the MAGUK protein PSD95 has been identified as a common outcome of DA receptor over stimulation in four different pharmacological or genetic models of altered monoamine neurotransmitter functions (Yao et al., 2004). In these models, a reduction of PSD95 expression is associated with altered synaptic plasticity in the striatum and with behavioral sensitization to DA drugs. Fumagalli et al. (2008) report that long-term haloperidol also prevents the normal trafficking of PSD95 to the PSDs but does not affect its expression.

Taken together, the findings of Fumagalli et al. (2008) reveal that long-term administration of FGAs induces a deficit in the trafficking of glutamate ionotropic receptors and their associated regulatory proteins to PSD in the frontal cortex (Fig. 1). This would result in a reduction of both AMPA and NMDA receptor synaptic receptor functions in this brain region and in the development of cognitive impairments in response to haloperidol. Theses findings support previous reports that long-term haloperidol treatment induces working memory impairments in nonhuman primates (Castner et al., 2000). It is noteworthy that haloperidol had no effect on the trafficking of glutamate receptor subunits in the hippocampus, thus suggesting that its negative effect on cognition may be restricted to a subset of brain regions. This region specificity may provide a way out of an apparent paradox between the authors' observation that an FGA interferes with ionotropic glutamate receptor trafficking and the postulated implication of reduced NMDA receptor functions in the pathology of schizophrenia.

The results also suggest that olanzapine and potentially other SGAs do not reduce the trafficking of glutamate receptor complexes to PSD in the frontal cortex. This may provide a molecular basis for the reported clinical superiority of some SGAs in addressing cognitive deficits in schizophrenia. Furthermore, because AMPA and NMDA are also involved in the regulation of motor functions, alterations of glutamate receptor trafficking by FGAs may also contribute to their enhanced propensity to induce extrapyramidal side effects (Gardoni et al., 2008). However, before any definitive conclusions, the study would have to be extended to include multiple different FGAs and SGAs tested at multiple doses. This can be particularly important because recent clinical evidences have shown that when given at low doses, haloperidol induces fewer side effects but still exerts its therapeutic action in patients with schizophrenia (Oosthuizen et al., 2004).

Another question raised by these results is the mechanism by which haloperidol regulates ionotropic glutamate trafficking. Haloperidol is believed to exert most of its effect by acting as a potent DA D2-class receptor antagonist (Creese et al., 1976). These receptors are coupled to at least two independent signaling pathways (Fig. 1) in the adult brain (Beaulieu et al., 2007). A first pathway involves the activation of $G\alpha_{i/o}$ G proteins, which results in the regulation of ion channels and in the inhibition of adenylate cyclases (Missale et al., 1998). Blockade of this pathway by haloperidol increases the production of cAMP leading to an activation of PKA. It is noteworthy that both NMDA and AMPA receptors contain PKA phosphorylation sites that have been shown to regulate their function and trafficking (Esteban et al., 2003).

D2-class receptors also regulate the Akt/GSK-3 signaling pathway (Fig. 1) through a cAMP-independent mechanism (Beaulieu et al., 2004). Upon activation, D2 receptors induce the formation of a signaling complex composed of the protein kinase Akt, the scaffolding protein β-arrestin 2, and protein phosphatase 2A (PP2A). The formation of this complex facilitates the inactivation of Akt by PP2A (Beaulieu et al., 2005). Because Akt is a negative regulator of GSK-3, inhibition of Akt in response to D2 receptor stimulation leads to an activation of GSK-3 (Beaulieu et al., 2008). It is noteworthy that recent evidence has implicated GSK-3 in the regulation of long-term plasticity in hippocampal slice preparation, thus suggesting a potential role for this kinase in the regulation of ionotropic glutamate receptor functions (Peineau et al., 2007).

Finally, regulation of glutamate receptor trafficking may also be independent from D2 DA receptors. For instance, coadministration of DA D1 receptor agonists can prevent the development of short-term memory deficit in response to long-term haloperidol in nonhuman primates (Castner et al., 2000). Because haloperidol can act as weak D1 receptor antagonists (Roth et al., 2004), it is possible that regulation of ionotropic glutamate receptor trafficking by D1 receptors (Cepeda and Levine, 2006) may also explain some of the effects observed by Fumagalli et al. (2008).

In summary, the article by Fumagalli et al. (2008) provides new avenues to examine the differential effects and mechanism of action of two important classes of antipsychotic drugs used in the management of schizophrenia and other neuropsychiatric conditions. Furthermore, the authors have shown that a single drug, haloperidol, can exert different effects on ionotropic glutamate receptors between the frontal cortex and the hippocampus. Such differences between brain regions suggests possible differences in the regulation of ionotropic glutamate receptor trafficking between neuronal cell types and underscores the importance of conducting molecular studies in vivo.

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