Antipsychotic medication is often associated with adverse effects, such as extrapyramidal symptoms (EPS) and increased serum prolactin. There is evidence to suggest that the lower liability to produce EPS and increased prolactin attributed to newer, so-called atypical antipsychotics, is correlated with their faster rates of dissociation from the dopamine D2 receptor [1]. Recent studies have indicated that the novel D2 receptor ligands, ACR16 and (-)-OSU6162, initially described as “dopamine stabilizers,” act as antagonists (alternatively, very weak partial agonists), with similarly high dissociation rates [2, 3]. However, these previous studies of antagonist unbinding rates measured either dissociation of radiolabeled ligand from membrane preparations [1] or used modified G proteins to study receptor activation-induced calcium release in living cells [2, 3]. We wanted to examine the relative kinetics of antagonist dissociation in living cells, using an assay based on activation of G protein-coupled potassium (GIRK) channels. This assay uses native G proteins and has higher temporal resolution than previously used assays.

Investigation of antagonist unbinding from dopamine D2 receptors using a time-resolved ion channel activation assay

Kristoffer Sahlholm, Sofia Frisk, Johanna Nilsson, Daniel Marcellino, Sven Ove Ögren, Kjell Fuxe, Peter Århem

Dept. of Neuroscience, Karolinska Institutet, SE-171 77, Stockholm, Sweden
Email: kristoffer.sahlholm@ki.se

Abstract

Antipsychotic medication is often associated with adverse effects, such as extrapyramidal symptoms (EPS) and increased serum prolactin. There is evidence to suggest that the lower liability to produce EPS and increased prolactin attributed to newer, so-called atypical antipsychotics, is correlated with their faster rates of dissociation from the dopamine D2 receptor [1]. Recent studies have indicated that the novel D2 receptor ligands, ACR16 and (-)-OSU6162, initially described as “dopamine stabilizers,” act as antagonists (alternatively, very weak partial agonists), with similarly high dissociation rates [2, 3]. However, these previous studies of antagonist unbinding rates measured either dissociation of radiolabeled ligand from membrane preparations [1] or used modified G proteins to study receptor activation-induced calcium release in living cells [2, 3]. We wanted to examine the relative kinetics of antagonist dissociation in living cells, using an assay based on activation of G protein-coupled potassium (GIRK) channels. This assay uses native G proteins and has higher temporal resolution than previously used assays.

Investigation of dopamine D2 receptor antagonism using the GIRK assay: Differential potencies

Response recovery from inhibition: Ligand-specific time course and extent of recovery

Faster recovery from block suggests faster dissociation from receptor

Pre-application of faster-dissociating antagonist remoxipride speeds up recovery following clozapine application, suggesting action at the same site at least for the recoverable fraction of clozapine antagonism.

Response recovery from inhibition: Summarized data

Time to half-maximal reactivation (T1/2) and relative reactivation amplitude, expressed as fraction of initial response, were taken as measures of antagonist dissociation from the receptor. No appreciable reactivation was observed upon washout of haloperidol, risperidone, paliperidone, bifeprunox and aripiprazole.

Time to half-maximal reactivation (T1/2) and relative reactivation amplitude following application of clozapine with and without preapplication of remoxipride.

Methods

Xenopus oocytes were injected with cRNA encoding the human dopamine D2 receptor, RGS-4, and GIRK14 channel subunits. RGS-4 is a GTPase accelerating protein typically present in native cells, which speeds up the G protein cycle, such that GIRK channel activity more closely follows receptor occupancy by agonist. GIRK current responses to dopamine receptor antagonist application were recorded at ~80 µM using two-electrode voltage clamp, as previously described [4].

References


The authors declare no potential conflict of interest.