Investigation of antagonist unbinding from dopamine D_2 receptors using a time-resolved ion channel activation assay

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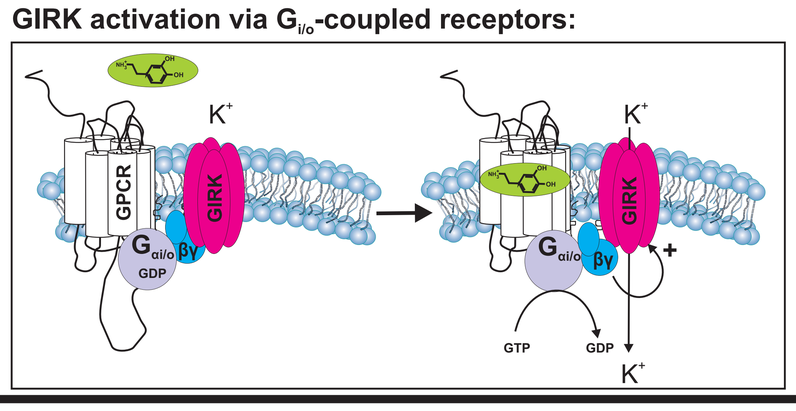
Abstract

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 D_2 receptor [1]. Recent studies have indicated that the novel D_2 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.

Assay principles

First, to establish the relevant working concentrations for the various compounds under study, concentration-response data for inhibition of D_2 receptor-stimulated GIRK channel opening were obtained. A baseline (100 nM dopamine) response was elicited, followed by 4-5 consecutive applications of test compound, each of 50 s duration, in the continued presence of dopamine. Under the conditions used, all test compounds showed negligible efficacy, and will hence be referred to as antagonists in the present context.

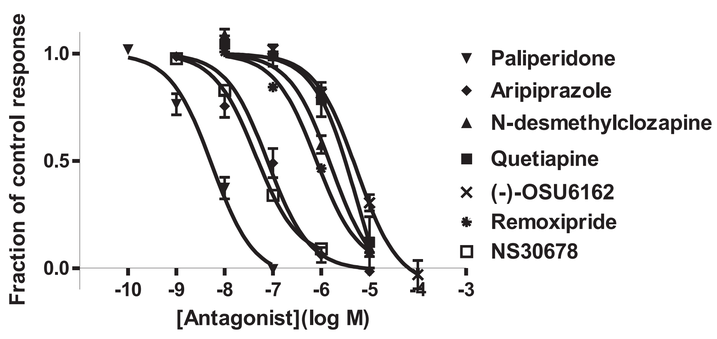
GIRK activation via $G_{i/o}$-coupled receptors:

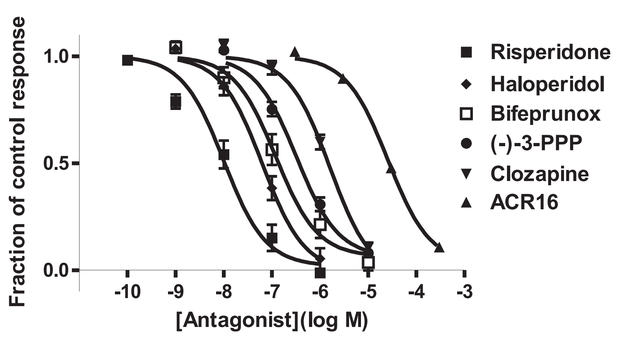


Second, the speed of receptor reactivation upon antagonist washout was measured, based on the assumption that faster dissociating antagonists should allow for faster reactivation of the receptor, and vice versa. For determination of reactivation kinetics upon antagonist washout, dopamine (100 nM) was applied first, resulting in a baseline GIRK response. Next, a near maximally effective concentration of antagonist was washed in, in the continued presence of dopamine. After a steady state of response inhibition had been established, the antagonist was washed out, still in the presence of dopamine. Response recovery was recorded over six minutes, and the recovery time-course and the maximal amplitude of the (pseudo)-steady state current relative to the baseline response were taken as relative measures of antagonist dissociation.

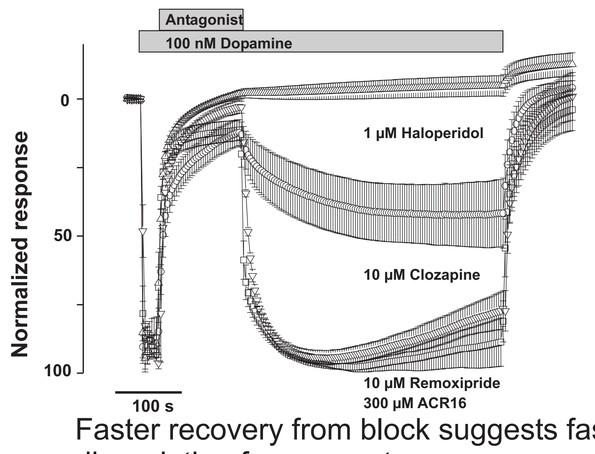
Results

Investigation of dopamine D_2 receptor antagonism using the GIRK assay: Differential potencies

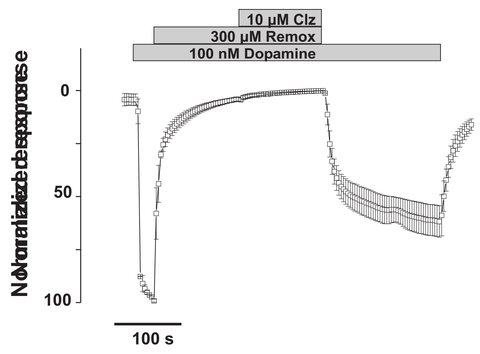




Response recovery from inhibition: Ligand-specific time course and extent of recovery

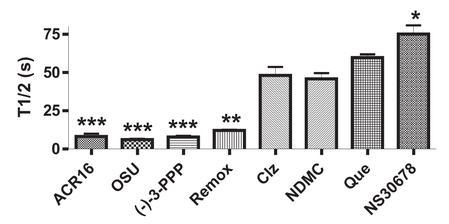


Faster recovery from block suggests faster dissociation from receptor

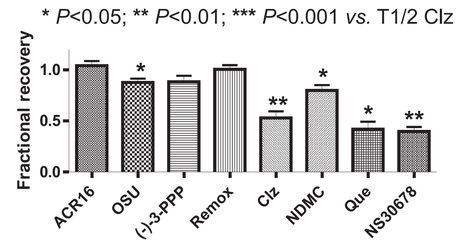


Pre-application of faster-dissociating antagonist remoxipride speeds up recovery following clozapine application, suggesting action at the same site at least for the recoverable fraction of clozapine antagonism

Response recovery from inhibition: Summarized data

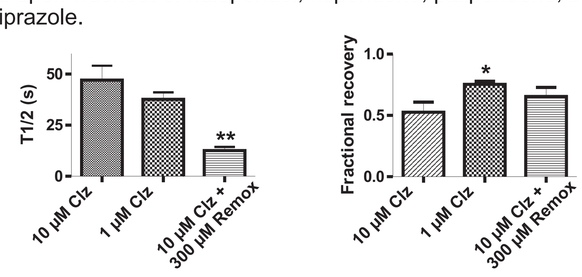


* P<0.05; ** P<0.01; *** P<0.001 *vs.* T1/2 Clz



* P<0.05; ** P<0.01; signficantly different from 1

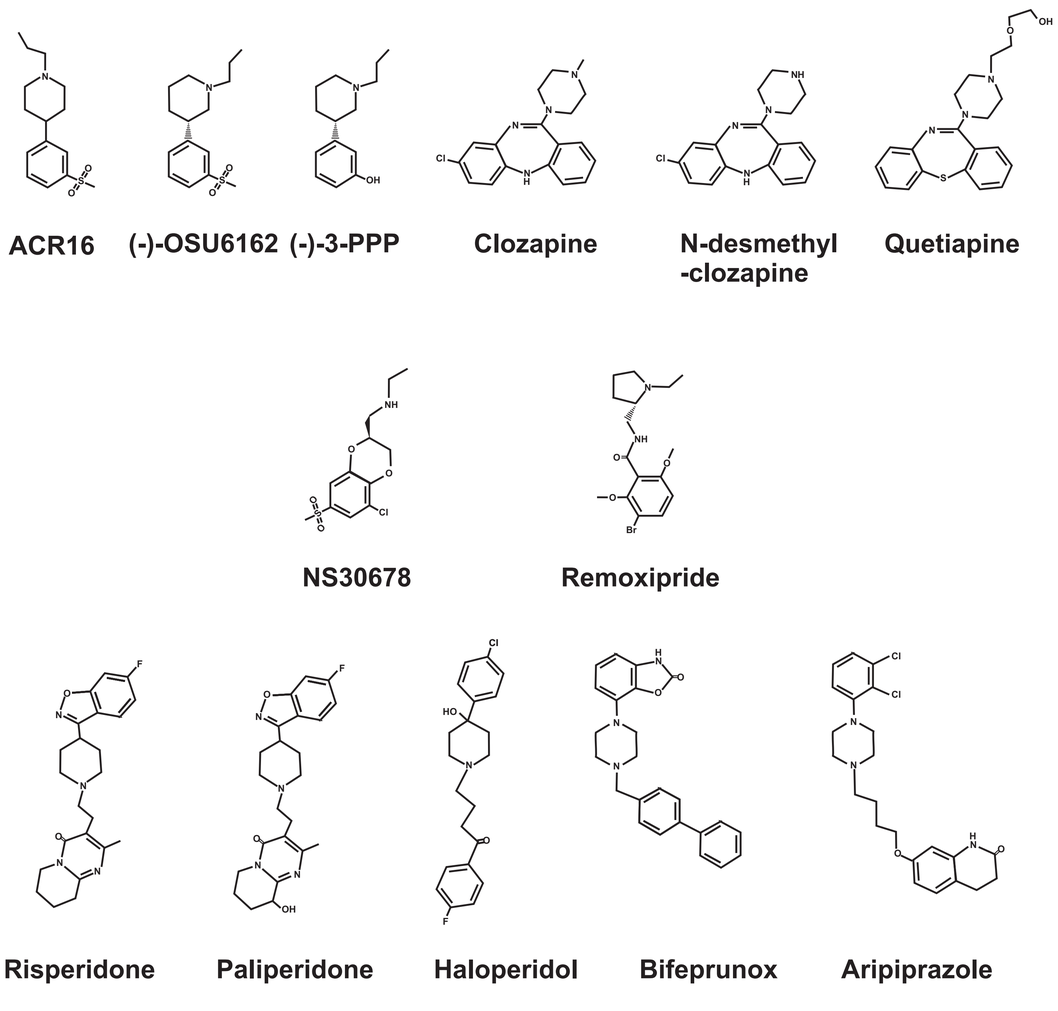
Time to half-maximal reactivation (T1/2) and relative reactivation amplitude, expressed as fraction of initial response, were taken as measures of antagonist dissociation from the receptor. No appreciable reactivation was observed upon washout of haloperidol, risperidone, paliperidone, bifeprunox and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



* P<0.05; ** P<0.01 *vs.* 10 µM Clz

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

Compound structures



Summary

- High temporal resolution of GIRK assay reveals faster response recovery on washout of the novel "dopamine stabilizers" (-)-OSU6162 and ACR16, as well as the benzamide antipsychotic remoxipride, compared to clozapine analogues – relevance to favorable side-effect profiles?

- Response recovery levels off at submaximal amplitude for clozapine and quetiapine – receptor internalization, desensitization, or binding site heterogeneity?

- 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

- Results confirm and extend earlier findings of faster response recovery/dissociation with clozapine analogues *vs.* haloperidol and risperidone

Methods

Xenopus oocytes were injected with cRNA encoding the human dopamine D_{2S} receptor, RGS-4, and GIRK1/4 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 activation were recorded at -80 mV using two-electrode voltage clamp, as previously described [4].

References

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The authors declare no potential conflict of interest.