Antipsychotics with similar association kinetics at dopamine D₂ receptors differ in extrapyramidal side-effects

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The recent publication in *Nature Communications* by Sykes et al. made the interesting observation that forward binding rate constants (k₄) at the dopamine D₂ receptor (D₂R) differ widely between antipsychotics, and moreover, that k₄ rather than k₂ (dissociation rate constant) values correlate with their liabilities to produce extrapyramidal symptoms (EPS). A high k₄ is postulated to cause a high degree of rebinding of the antipsychotic to D₂R, conferring increased competition with endogenous dopamine, and thereby more EPS. As pointed out by the authors, their results are largely in agreement with our previous findings from a potassium channel (G protein-activated inward rectifier potassium; GIRK) activation assay. However, there are some discrepancies between their work and ours which may have important consequences for the interpretation of the correlation between k₄ and EPS, and which we would like to point out below.

Remoxipride, a clinically effective antipsychotic which was withdrawn due to the occurrence of aplastic anemia as a rare side-effect, has a very low rate of EPS compared to the typical antipsychotic, haloperidol (odds ratio for rigidity, 0.30; 95% CI, 0.22 to 0.41; n = 1104; P < 0.0001, Fisher’s exact test; Fig. 1). Importantly, unlike many other antipsychotics which target multiple types of monoamine receptors, both of these compounds preferentially bind to dopamine D₂-like receptors. Thus, the difference in EPS liability between remoxipride and haloperidol cannot easily be attributed to differential engagement of muscarinic or serotonergic receptors, which are thought to contribute to the low EPS potential of many of the newer antipsychotics, such as clozapine, quetiapine, and olanzapine. This should make remoxipride and haloperidol particularly suitable for comparison based on their kinetic properties measured for remoxipride may have been distorted by the relatively slow kinetics of PPHT-red. In addition, the differences in kinetic parameters measured for remoxipride may have been distorted by the relatively slow kinetics of PPHT-red. In addition, the differences in kinetic parameters measured for remoxipride may have been distorted by the relatively slow kinetics of PPHT-red. In addition, the differences in kinetic parameters measured for remoxipride may have been distorted by the relatively slow kinetics of PPHT-red. In addition, the differences in kinetic parameters measured for remoxipride may have been distorted by the relatively slow kinetics of PPHT-red.
that drug interactions with the functional state of the receptor might be more relevant to the occurrence of side-effects. Whereas dopamine binding to D2R is known to be sensitive to the transmembrane voltage, such that the IC50 for dopamine-induced GIRK activation is about 4.2-fold higher at 0 mV compared to −80 mV, we found the IC50 of remoxipride to inhibit the GIRK response to 100 nM dopamine to be 4.8-fold lower at 0 mV (IC50 202.2 nM; pIC50 6.69 ± 0.11; n = 5) compared to −80 mV (IC50 963.5 nM; pIC50 6.02 ± 0.09; n = 5). The IC50 value at −80 mV has been published previously. This would be consistent with a competitive interaction between dopamine and remoxipride, where the affinity of remoxipride itself is not substantially altered by transmembrane voltage. Thus, a difference in remoxipride binding kinetics related to differential membrane potentials (the transmembrane voltage across isolated membranes, as used by Sykes et al., would be 0 mV, while the whole cells used in our study were clamped at a more physiological −80 mV), would seem unlikely, although a reciprocal change in both k on and k off cannot be ruled out. Lastly, we note that thiordiazine, which had been placed in the uncertain typical/atypical category, has among the highest k on values reported by Sykes et al., yet it has consistently been reported to have a favorable profile in terms of EPS.

In summary, while we find the concept of rebinding, as discussed by Sykes et al., to be of great interest and of potential utility to the field of neuropharmacology, we believe that k on is not the only factor governing EPS liability, even when considering D2R-prefering compounds. This is illustrated by comparing haloperidol and remoxipride, which despite having similar k on at D2R in our hands, show very distinct EPS profiles in the clinic.

**Methods**

**Electrophysiology data acquisition.** The IC50 of remoxipride at −80 and 0 mV was determined using two-electrode voltage clamp in Xenopus laevis oocytes expressing the human D2R together with RGS-4 and GIRK1/4 channels, measuring the GIRK current amplitude as a readout of D2R occupancy by dopamine. For concentration-response data, oocytes were randomly clamped at either −80 or 0 mV and exposed first to 100 nM dopamine, to evoke GIRK activation. Thereafter, four increasing concentrations (10 nM–10 µM) of remoxipride were applied consecutively, in the continued presence of 100 nM dopamine, at 50-s intervals. The current amplitude at the end of each antagonist application interval was normalized to the control response to 100 nM dopamine, within every oocyte. k on values were also obtained using the GIRK assay, and were taken from our previous work. Oocyte harvesting from female Xenopus laevis toads was performed in accordance with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health. Prior approval for the procedure was granted by the Swedish National Board for Laboratory Animals and the local ethics committee, Stockholm's Norra Djurförsöksnämndet.

**Odds ratio calculation.** The odds ratio for remoxipride vs. haloperidol was obtained from EPS (rigidity) incidences and number of patients reported by Lewander et al. The odds ratio for remoxipride vs. placebo was calculated as

$$OR(P) = \frac{OR_{R_{EPS}}}{OR_{H_{EPS}}}$$

(1)

where OR R_{EPS} and OR H_{EPS} denote number of patients treated with remoxipride, haloperidol, and placebo, with our with or without EPS, respectively. Given the standard error SE of OR calculated from the data set included remoxipride did not use the Bayesian hierarchical model employed by Leucht et al. However, the odds ratio calculated here should provide a good estimate for comparison with the antipsychotics in the original analysis.

**Statistical analysis.** The statistical significance of the differential proportions of patients experiencing EPS between haloperidol-treated and remoxipride-treated subjects (data from Lewander et al.) was calculated using Fisher’s exact test.

**Data availability.** All relevant data are available from the authors upon request.

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HZ. performed the mathematical analysis. K.S. drafted the first version of the manuscript. Both authors jointly conceived of the idea for the paper and prepared the final version of the manuscript.

Additional information
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