Phenothiazine-Derived Antipsychotic Drugs Inhibit Dynamin and Clathrin-Mediated Endocytosis


Abstract:

Chlorpromazine is a phenothiazine-derived antipsychotic drug (APD) that inhibits clathrin-mediated endocytosis (CME) in cells by an unknown mechanism. We examined whether its action and that of other APDs might be mediated by the GTPase activity of dynamin. Eight of eight phenothiazine-derived APDs inhibited dynamin I (dynI) in the 2–12 µm range, the most potent being trifluoperazine (IC50 2.6 ± 0.7 µm). They also inhibited dynamin II (dynII) at similar concentrations. Typical and atypical APDs not based on the phenothiazine scaffold were 8- to 10-fold less potent (haloperidol and clozapine) or were inactive (droperidol, olanzapine and risperidone). Kinetic analysis showed that phenothiazine-derived APDs were lipid competitive, while haloperidol was uncompetitive with lipid. Accordingly, phenothiazine-derived APDs inhibited dynI GTPase activity stimulated by lipids but not by various SH3 domains. All dynamin-active APDs also inhibited transferrin (Tfn) CME in cells at related potencies. Structure/activity relationships (SAR) revealed dynamin inhibition to be conferred by a substituent group containing a terminal tertiary amino group at the N2 position. Chlorpromazine was previously proposed to target AP-2 recruitment in the formation of clathrin-coated vesicles (CCV). However, neither chlorpromazine nor thioridazine affected AP-2 interaction with amphiphysin or clathrin. Super-resolution microscopy revealed that chlorpromazine blocks neither clathrin recruitment by AP-2, nor AP-2 recruitment, showing that CME inhibition occurs downstream of CCV formation. Overall, potent dynamin inhibition is a shared characteristic of phenothiazine-derived APDs, but not other typical or atypical APDs, and the data indicate that dynamin is their likely in-cell target in endocytosis.

Published In:

Traffic, Vol 16, No 6, 635–654