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Antipsychotics inhibit TREK but not TRAAK channels

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Abstract

Schizophrenia is a chronic mental illness affecting 0.4% of the population. Existing antipsychotic drugs are mainly used to treat positive symptoms such as hallucinations but have only poor effects on negative symptoms such as cognitive deficits or depression. TREK and TRAAK channels are two P domain background potassium channels activated by polyunsaturated fatty acids and mechanical stress. TREK but not TRAAK channels are regulated by Gs- and Gq-coupled pathways. The inactivation of the TREK-1 but not the TRAAK channel in mice results in a depression-resistant phenotype. In addition, it has been shown that antidepressants such as fluoxetine or paroxetine directly inhibit TREK channel activity. Here we show that different antipsychotic drugs directly inhibit TREK currents with IC_{50} values of ~1 to ~20 μM. No effect is seen on TRAAK channel activity. We conclude that TREK channels might be involved in the therapeutic action of antipsychotics or in their secondary effects. Furthermore, TREK channels could play a role in the pathophysiology of psychiatric disorders such as depression and schizophrenia.

Keywords: Two-pore domain background potassium channels; Mental disorders; Neuroleptics; Schizophrenia; Depression

Schizophrenia is a chronic serious mental illness marked by episodes of exacerbated psychotic symptoms such as hallucination and delusion or chronic disability of mental and social functions. It affects 0.4% of the population [1]. Treatment of schizophrenia remains difficult and often inadequate in many patients despite the discovery of chlorpromazine as the first 'typical' antipsychotic in the 1950s and the development of 'atypical' antipsychotics like clozapine 20 years later.

Existing antipsychotics are mainly used to treat positive symptoms of schizophrenia such as hallucination, delusion, and aggression, but have only a poor effect on negative symptoms such as cognitive deficits, depression or social disabilities. In addition, 30–60% of acutely ill patients fail to respond or respond inadequately to existing drugs [2,3]. A major side effect of 'typical' antipsychotics is extrapyramidal symptoms such as parkinsonism, dystonia or tardive dyskinesia as a consequence of dopamine D2 receptor antagonism. Treatment with 'atypical' antipsychotics which interact in addition with serotonin, muscarinic, cholinergic, and histamine receptors is complicated by weight gain, diabetes, cardiovascular, and hematological side effects [3].

Potassium channels constitute the biggest and most diversified family of ion channels. Mutations of K^+ channels in humans are resulting in a number of channelopathies touching brain, muscle, heart, and other organs. TREK and TRAAK channels belong to the two P domain background potassium channel family (K_{2P}) (for reviews, see [4–7]). They produce outwardly rectifying currents activated by polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA), intracellular acidification, and mechanical stress. TREK and TRAAK channels are expressed throughout the central nervous system and are stimulated by volatile anesthetics [8] and by the neuroprotective drug riluzole [9]. Furthermore, the TREK channel is

Abbreviations: AA, arachidonic acid; K^+, potassium; K_{2P}, two P domain potassium channel; KO, knock-out; PUFA, polyunsaturated fatty acid; SSRI, selective serotonin reuptake inhibitor.

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the probable homologue of the Aplysia S-type potassium channel involved in a simple form of learning and memory [8,10]. The closely related TREK-1 and TREK-2 channels are regulated by phosphorylation by protein kinase A and C [11–13] and can therefore be modulated by neurotransmitters and hormones acting via Gs- and Gq-coupled receptors. On the contrary, the TRAAK channel is lacking these particular regulations [14].

The invalidation of the TREK-1 gene (knock-out) results in a depression-resistant phenotype related to an increased level of serotoninergic neurotransmission [15]. In addition, TREK-1 knock-out mice loose neuroprotection by PUFAs and are more vulnerable to brain ischemia [16].

TREK channels are inhibited by selective serotonin reuptake inhibitors (SSRIs) such as paroxetine and fluoxetine with an IC_{50} value in a ~3–10 μM range [15,17]. In contrast, the TRAAK channel is insensitive to these classical antidepressants. Interestingly, TRAAK KO mice do not show the antidepressant phenotype of TREK-1 KO mice [15]. Furthermore, brain concentrations of SSRIs in patients treated for depression are in the same range as those necessary to antagonize TREK channels [15]. In addition to antidepressants, the antipsychotic drug chlorpromazine has been shown to antagonize TREK-1 currents [8].

All these observations led us to further investigate the effect of antipsychotics on background potassium channels of the TREK family. The observed inhibitory action of antipsychotics on TREK but not TRAAK channels in therapeutically relevant drug concentrations suggests an implication of the TREK channel not only in the pathophysiology of depression but also in other psychiatric disorders such as schizophrenia.

Methods

COS cells were seeded at a density of 20,000 cells/35-mm dish 24 h before transfection. Cells were transiently transfected by the classical DEAE-dextran method with hTREK-1, hTREK-2 or hTRAAK plasmids and co-transfected with EYFP, a fluorescent marker. Transfected cells were visualized 48–72 h after transfection using fluorescence.

For whole-cell and outside-out experiments the bath solution (EXT) contained (in mM): 150 NaCl, 5 KCl, 1 $CaCl_2$, 3 $MgCl_2$, and 10 Hepes, adjusted to pH 7.4 with NaOH; the patch pipette solution (INT) contained (in mM): 155 KCl, 3 $MgCl_2$, 5 EGTA, and 10 Hepes, adjusted to pH 7.2 with KOH. For inside-out recordings, the bath solution was INT and pipettes were filled with EXT. Patch-clamp pipettes were made from borosilicate and had a resistance of 1.8–3 MΩ. Cells were clamped at −80 mV and voltage changes were either applied by ramp (from −100 to +50 mV, 1 s in duration) or by step (from −100 to +40 mV, 1.8 s in duration). Cells were continuously superfused with a microperfusion system. Application of the same pharmacological agent in different concentrations always started with the lowest concentration. Recordings were done at room temperature (21–22 °C) using a MultiClamp 700A computer-controlled patch-clamp amplifier (Axon Instruments, USA). PClamp software was used to analyze recorded data. Currents recorded in the whole-cell mode were measured at 0 mV. All results are expressed as means ± SEM, with *n* indicating the numbers of cells tested. To obtain IC_{50} values for concentration-dependent inhibition experimental data were averaged and then fitted with a standard sigmoidal function.

All pharmacological reagents were obtained from Sigma. Stock solutions were prepared in H_2O, ethanol or DMSO and kept at −20 °C. DMSO and ethanol were never exceeding 1% of final solution.

Results

In a first set of experiments we studied the effect of antipsychotic drugs on whole-cell TREK-1 currents. We tested different antipsychotics which can be classified by their chemical structure.

All but *substituted benzamides* significantly inhibited whole-cell TREK-1 currents at a concentration of 10 μM (Figs. 1A and 4). Inhibition of TREK-1 currents was not voltage-dependent and did not result in a modification of current kinetics (Fig. 1A). The *phenothiazines* fluphenazine and chlorpromazine (10 μM) inhibited human TREK-1 currents by 64 ± 8% and 83 ± 2%, the *butyrophenone* haloperidol by 59 ± 6%, and the *thioxanthene* flupenthixol by 84 ± 6%. The *dibenzo-oxazepine* loxapine and the *dibenzodiazepine* clozapine inhibited TREK-1 currents by 37 ± 4% and 52 ± 5% and pimozide by 91 ± 4%. The *substituted benzamides* sulpiride and tiapride had no inhibitory action on TREK-1 (107 ± 4% and 116 ± 7% of control current; Fig. 4, the number of cells tested is indicated in the figure).

Antipsychotics resulted in a concentration-dependent and reversible inhibition of TREK-1 whole-cell currents (Fig. 2). IC_{50} values for the inhibition of TREK-1 currents varied from 1.8 to 19.7 μM (fluphenazine 4.7 ± 1.6 μM, $n = 13$; chlorpromazine 2.7 ± 0.3 μM, $n = 6$; haloperidol 5.5 ± 0.7 μM, $n = 6$; flupenthixol 2.0 ± 0.5 μM, $n = 12$; loxapine 19.7 ± 2.7 μM, $n = 12$; pimozide 1.8 ± 0.3 μM, $n = 9$).

In further experiments we tested the hypothesis that the inhibitory action of antipsychotics on TREK-1 currents is by a direct action on TREK-1 channels and not via intracellular mechanisms as activation of phosphorylation pathways. We applied the antipsychotic fluphenazine (10 μM) directly on the cell membrane in excised patches of TREK-1-transfected COS cells. Fluphenazine had an inhibitory action on TREK-1 inside-out currents in all patches tested ($n = 4$, a representative example is shown in Fig. 1B).

Are antipsychotics modulating other lipid- and mechanogated K_{2P} channels in addition to TREK-1? To answer this question we studied the action of antipsychotic agents on human TREK-2 and TRAAK currents. TREK-2 currents were, as TREK-1 currents, inhibited by the majority of antipsychotics at a concentration of 10 μM (Figs. 3A and 4). Fluphenazine inhibited 77 ± 6% of control current, chlorpromazine 89 ± 2%, haloperidol 51 ± 5%, flupenthixol 82 ± 5%, loxapine 51 ± 5%, clozapine 50 ± 5%, and pimozide 88 ± 5%. The *substituted benzamides* sulpiride and tiapride did not antagonize TREK-2 currents (102 ± 1% and 121 ± 3% of control current). Inhibition of TREK-2 currents was concentration-dependent and reversible. IC_{50} values for the inhibition of TREK-2 currents varied from 1.3 to

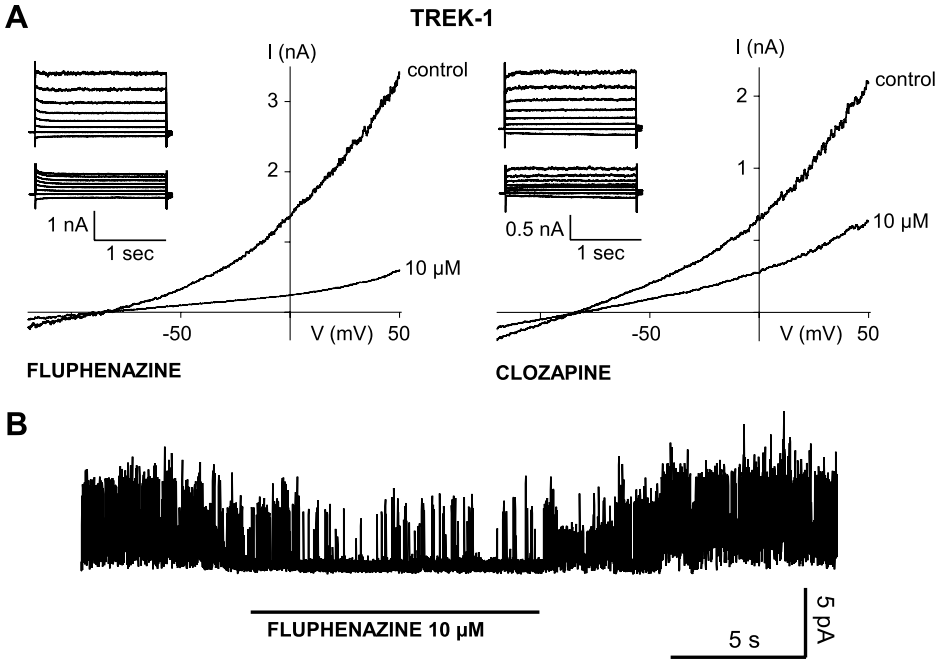

Fig. 1. Antipsychotics inhibit human TREK-1 currents. (A) Whole-cell currents were evoked by voltage ramp and steps in absence (control) and in presence of the 'typical' and 'atypical' antipsychotics fluphenazine and clozapine (10 μM). (B) Fluphenazine inhibits reversibly inside-out TREK-1 currents. Single-channel currents were recorded continuously at +40 mV. Channel openings correspond to an upward direction.

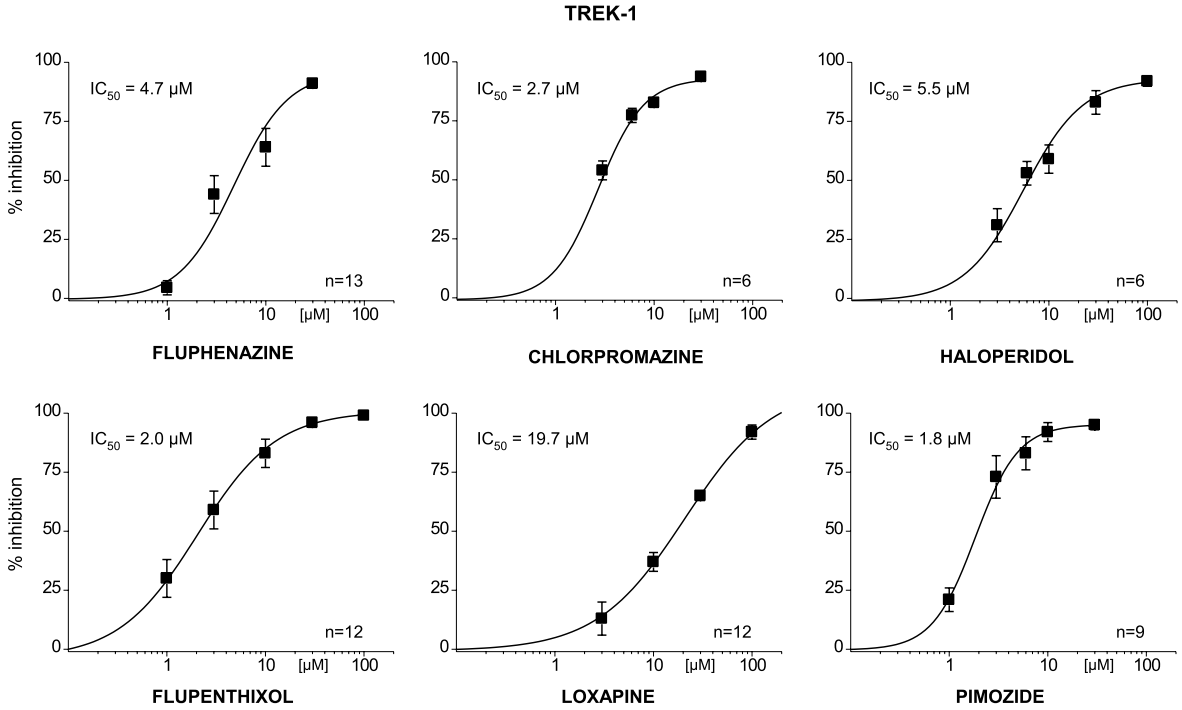

Fig. 2. Antipsychotics result in a complete, dose-dependent inhibition of human TREK-1 currents. Dose–response curves for different antipsychotic drugs are represented in the graphs with IC_{50} values ranging from ~2 to ~20 μM. Currents were measured at 0 mV.

8.5 μM (fluphenazine 2.5 ± 0.5 μM, $n = 7$; chlorpromazine 2.6 ± 0.1 μM, $n = 5$; haloperidol 6.3 ± 1.2 μM, $n = 6$; fluphenthixol 1.3 ± 0.1 μM, $n = 9$; loxapine 8.5 ± 2.6 μM, $n = 9$; pimozide 3.3 ± 0.9 μM, $n = 6$). In addition, we observed an inhibitory action of different antipsychotics on TREK-2 inside-out currents in excised patches (haloperidol 30 μM, $n = 4$; clozapine 30 μM, $n = 3$; loxapine 30 μM, $n = 4$). A representative example is shown in Fig. 3B.

In contrast to TREK currents, TRAAK currents were not inhibited by antipsychotic drugs at a concentration of

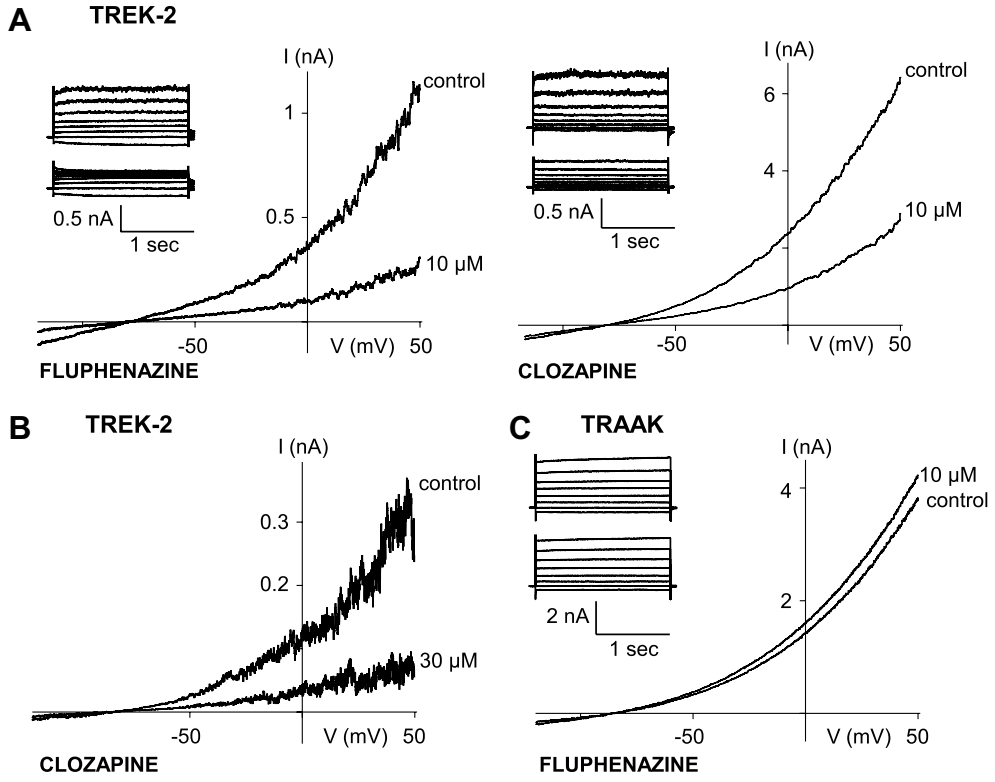

Fig. 3. Antipsychotic drugs block human TREK-2 currents which are closely related to TREK-1 but have no effect on TRAAK currents. (A) TREK-2 whole-cell currents were evoked by voltage ramp and steps in absence (control) and in presence of the 'typical' and 'atypical' antipsychotics fluphenazine and clozapine (10 μM). (B) Clozapine (30 μM) inhibits inside-out TREK-2 currents elicited by voltage ramps. (C) Fluphenazine has no effect on TRAAK whole-cell currents evoked by voltage ramp and steps.

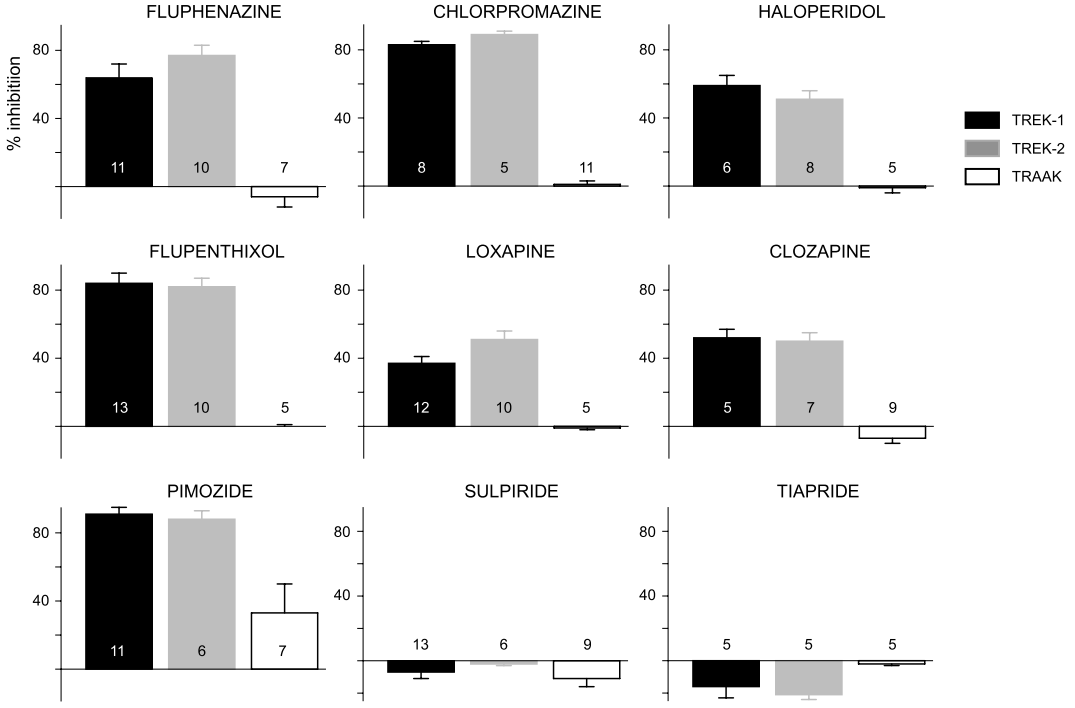

Fig. 4. All antipsychotics tested with exception of the *substituted benzamides* sulpiride and tiapride inhibit whole-cell human TREK-1 and TREK-2 but not TRAAK currents at a drug concentration of 10 μM. The number of cells tested is indicated.

10 μM (Figs. 3C and 4). Fluphenazine evoked 106 ± 6% of control current, chlorpromazine 99 ± 2%, haloperidol 101 ± 3%, flupenthixol 100 ± 1%, loxapine 101 ± 1%, clozapine 107 ± 3%, pimozide 67 ± 17%, sulpiride 111 ± 5%, and tiapride 102 ± 1% (Fig. 4).

Discussion

We have shown that the TREK-1 and TREK-2 background potassium channel can be inhibited by several 'typical' and 'atypical' antipsychotic drugs with IC_{50} values of ~1 to ~20 μM. The TRAAK channel is not inhibited by antipsychotics at drug concentrations that potently block TREK channel activity. TREK and TRAAK channels are close members of the K_{2P} channel family [3]. Both TREK and TRAAK channels are activated by polyunsaturated fatty acids and by membrane stretch. However, only TREK channels can be regulated through Gs- and Gq-coupled pathways [8,13,18].

Active brain concentrations of antipsychotics are difficult to assess. Several studies in rat suggest an accumulation of antipsychotics in brain tissue with brain to plasma ratios ranging from 7:1 to 35:1 and corresponding to a brain drug concentration of 1–5 μM [19–23]. In humans, studies of antipsychotic brain concentrations are rare and brain concentrations as high as 3–30 μM are reported [24–26]. Therefore, inhibition of the TREK channel might be expected at therapeutically relevant drug concentrations.

It has been shown that TREK-1 but not TRAAK channels are inhibited by SSRIs like paroxetine and fluoxetine, drugs commonly used in the therapy of depression [15,17]. The inactivation of the TREK-1 channel in mice results in a depression-resistant phenotype. In addition to an increased serotoninergic neurotransmission in the TREK-1 KO mice, direct action of SSRIs on the TREK-1 channel in addition to the blockade of the serotonin transporter might be involved in the therapeutic action of these antidepressants or in their secondary effects [15,17].

Depression-like symptoms are common features in different psychiatric diseases such as schizophrenia or learning and memory disorders. Common symptoms of those diseases can be observed in patients with major depression. Therefore, similarities in the pathophysiology of psychiatric disorders might explain these observations. In addition, the SSRI paroxetine has been shown to be effective in treating negative symptoms when added to antipsychotic therapy in patients suffering from schizophrenia [27].

Our finding that TREK channels can be modulated by antipsychotic drugs in addition to antidepressants suggests that TREK channels might be involved in the therapeutic action of antipsychotics or in their secondary effects. Furthermore, TREK channels could play a role in the pathophysiology of psychiatric disorders such as depression and schizophrenia. Selective blockers of TREK channels might therefore be promising new drugs for the management of psychiatric diseases.

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